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Endoscopic mucosal resection with 0.13% hyaluronic acid solution for colorectal polyps less than 20 mm: A randomized controlled trial

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Abstract

Background and Aim: Adequate mucosal elevation by submucosal injection is important for definitive en bloc resection and prevention of perforation during endoscopic mucosal resection (EMR). The objective of this study is to determine the efficacy of 0.13% hyaluronic acid (HA) solution for high and sustained mucosal elevation during colorectal EMR.
Methods: The study was a prospective randomized controlled trial; a total of 196 patients with colon polyps of < 20 mm diameter were enrolled and randomized in a 1:1 ratio to undergo EMR using either 0.13% HA or normal saline (NS). The primary outcome of the study was histopathologically confirmed complete resection. The secondary outcomes such as maintenance of high mucosal elevation and development of complications were also evaluated. Moreover, the relationship between complete resection and the experience of the endoscopist (veteran *vs* less experienced) was analyzed.
Results: Compete resection was achieved in 74 of 93 polyps (79.5%) in the 0.13% HA group and 63 of 96 polyps (65.6%) in the NS group ($P < 0.05$). High mucosal elevation was maintained in 83.9% of procedures in the 0.13% HA group and 54.1% in the NS group ($P < 0.01$). The frequency of complete resection achieved by less-experienced endoscopists was higher in the 0.13% HA group (79.3%) than in the NS group (62.1%; $P < 0.05$).
Conclusions: Endoscopic mucosal resection using 0.13% HA to colon polyps of less than 20 mm diameter is more effective than NS for complete resection and maintenance of mucosal elevation.

Introduction

Endoscopic mucosal resection (EMR) is considered the standard procedure for colorectal polyps.[1] Rosenberg first described the saline injection-assisted method and identified it as a safety factor for removing rectal and sigmoid polyps, and this technique was reintroduced by Tada *et al.* in 1984.[2–4] Most adenoma and intramucosal cancers can be resected by EMR; however, tumors of > 20 mm diameter are considered difficult candidates for en bloc resection.[5,6] Endoscopic submucosal dissection (ESD) can remove large-sized lesions of early colorectal cancer, but it can be a time-consuming procedure and carries a higher risk of perforation than EMR.[7,8] Thus, it is important to improve the technical feasibility of EMR for en bloc and extended resections. Moreover, most colorectal polyps removed by EMR are < 20 mm diameter in size. The rate of en bloc resection and compete resection of EMR to these lesions is known to be good, but not perfect, especially for less experienced endoscopists. In EMR, submucosal fluid injection is necessary to obtain high mucosal elevation, which facilitates removal of the lesion. Low mucosal elevation makes snaring difficult; moreover, electrocautery damage of the muscularis propria due to low mucosal elevation may cause delayed perforation. We previously reported that mucosal elevation with normal saline (NS) dissipated within 2 min of injection, which is the length of time necessary for most endoscopists to perform EMR.[9] Many additional solutions have been used to achieve sustained mucosal elevation, definitive en bloc resection, and prevention of perforation in EMR. Hypertonic saline, glycerol, dextrose, fibrinogen, succinylated gelatin and autologous blood induce improvement of complete resection and mucosal elevation that lasts longer than that induced by NS.[10–14] Hyaluronic acid (HA) has been shown to create higher and sustainable mucosal elevation than NS.[15–17] Yamamoto *et al.* first reported the efficacy of HA in novel endoscopic resection of a large colorectal polyp, and this was subsequently termed ESD.[18] Injection of HA reportedly causes less tissue damage than hypertonic solutions such as dextrose, which

are known to cause damage at local injections sites.[19] However, the viscosity of high concentrations of HA can make snaring difficult in our previous study. It is important to dilute HA because HA is more expensive than NS.[9] Thus, we demonstrated that an HA concentration as low as 0.13% was effective for sustained mucosal elevation in resected porcine colon and in living minipig colon.[9] However, there is no clinical experience with EMR using 0.13% HA, and there have been no randomized controlled trials showing the efficacy of HA compared to NS in colorectal EMR. In the current study, we conducted a randomized controlled trial comparing the efficacy of 0.13% HA and NS in EMR to colorectal tumor of < 20 mm diameter.

Methods

The study was designed as a randomized controlled open trial, and was conducted at the Department of Molecular Gastroenterology and Hepatology at Kyoto Prefectural University of Medicine, Japan. Random assignment was performed using permuted blocks without stratification in a computer-generated random sequence. The inclusion criterion was the presence of neoplastic colorectal polyps of < 20 mm diameter that had been diagnosed using a double-contrast barium enema or precolonoscopy. If multiple lesions were present, only the biggest lesion was analyzed in this study. Exclusion criteria were recurrent lesions with previous EMR; lesions diagnosed as deeply submucosal invasive cancer by colonoscopy; non-neoplastic polyps; or pedunculated polyps. Only protruded and superficial colorectal polyps without a clear stalk were analyzed.[20] In detail, protruded type consisted of sessile type and subpedunculated type and superficial type consisted of elevated type, flat type and depressed type according to Japanese Classification of colorectal Carcinoma (JSCCR). The size of a polyp was defined as maximum diameter and was calculated referring to the size of snare and injection needle. If histopathological diagnosis of the polyp after EMR indicated a non-neoplastic polyp or a deeply submucosal invasive cancer, the polyp was excluded from the analysis. All the patients provided written informed consent for EMR, and the study was approved by the ethics committee of Kyoto Prefectural University of Medicine. This study was carried out in accordance with the World Medical Association Helsinki Declaration. This study has been registered in the University hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) as number UMIN000005457.

Endoscopic mucosal resection technique. We used a lower gastrointestinal endoscope with a single channel (EC-590MP; Fujifilm, Tokyo, Japan, and PCF-Q240ZI or PCF-Q260AZI; Olympus, Tokyo, Japan) and a video-endoscope system (Advancia VP-4450 V1.2; Fujifilm and EVIS LUCERA SPECTRUM; Olympus). The procedures were performed by seven endoscopists whose experience with colonoscopy was between 500 and 15 000 cases. The patient's bowels were prepared by consumption of 2 L of polyethylene glycol solution on the morning before examination. For the preparation of the injection solution, we used 0.4% HA solution (800 kDa preparation, Mucoup; Johnson & Johnson, Tokyo, Japan or Seikagaku Corporation, Tokyo, Japan), which was approved by the Japanese National Health Insurance System as the injection solution for EMR. The 0.13% HA solution was prepared by diluting 0.4% HA solution with NS. Both the 0.13% HA and NS solutions were injected using a 25-gauge needle (01885: TOP, Tokyo, Japan) and a 10-mL syringe. A small amount of indigo carmine was added to each solution to facilitate visualization of the mucosal elevation. Epinephrine was not added to the injection solutions. The EMR procedure time from injection to resection was recorded. The snare (15-mm SnareMaster, Olympus) was used with an automatically controlled high-frequency generator (VIO300D; Erbe Elektromedizin, Tübingen, Germany) set to the coagulation mode (swift coagulation, output 40 W, effect 3).

Histopathological evaluation. Histopathological diagnosis was based on the World Health Organization's classification.[21] All lesions were evaluated histopathologically by one pathologist (A.Y.). Adenoma was applied according to mild to severe cytological and architectural features. The "dysplasia" was defined as histopathologically unequivocal neoplastic epithelium without evidence of invasive growth. The "dysplasia" was thus only appropriate when cytological and/or architectural features of neoplasia were present. The "dysplasia" was classified to adenoma in the current study. The "intramucosal adenocarcinoma" was applied to lesions that show histological evidence of invasion into the lamina propria or muscularis mucosa but not into the submucosa. Complete resection was defined histopathologically by tumor-free lateral and vertical margins of the resected specimens.

Study outcomes. The primary endpoint of the study was complete resection and the secondary endpoints were maintenance of high mucosal elevation and associated complications. The state of mucosal elevation was examined before snaring and was classified into two macroscopic types: a maintained type in which mucosal elevation was maintained at more than 50% of the initial elevation and an unmaintained type in which mucosal elevation was maintained at less than 50% of the initial elevation (Figs 1,2). The evaluation of mucosal elevation was judged by two endoscopists; one was the endoscopist who performed EMR. The other, who did not know the content of injection solution, examined the evaluation of mucosal elevation using endoscopic figures. The maintenance of mucosal elevation was judged by the agreement of evaluation by these two endoscopists. Perforation was detected by endoscopy during the EMR procedure or by abdominal computed tomography after EMR. Postoperative hemorrhage was defined as the occurrence of hematochezia that required endoscopic treatment to stop the bleeding. The volume of injection solution, the EMR procedure time, and the rate of en bloc resection were evaluated as secondary endpoints. The en bloc resection was evaluated according to the agreement of two endoscopists to prevent the bias of objective evaluation by one endoscopist. The relationship between complete resection and polyp morphology, polyp size, or location was also evaluated as subgroup analyses. The analysis of polyp size was performed between polyp of 5–10 mm diameter and polyps of 11–20 mm. The polyp location was described as being from the cecum to the descending colon or from the sigmoid colon to the rectum. The relationship between complete resection and the experience of the endoscopist (less-experienced *vs* veteran endoscopist) was also evaluated as a

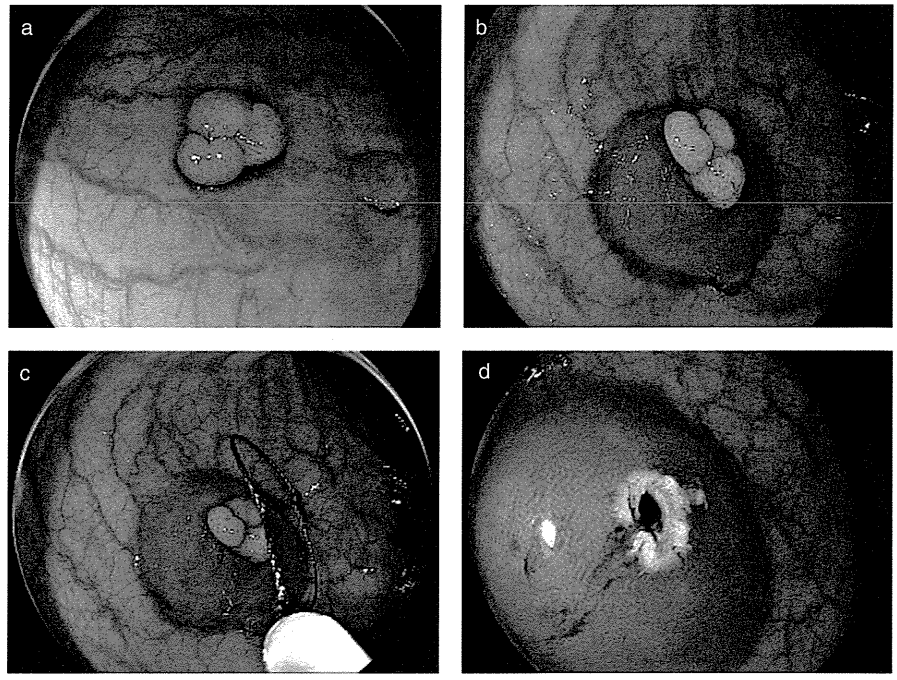

Figure 1 (a) A protruded polyp of 10 mm diameter in the rectum. (b) Initial high mucosal elevation was achieved after injection of 2.0 mL 0.13% hyaluronic acid (HA). (c) Maintenance of mucosal elevation was sufficient to allow snaring of the polyp. (d) The endoscopic mucosal resection (EMR) was accomplished with a procedure time of 110 s. Histopathological examination of the polyp showed adenoma and confirmed complete resection.

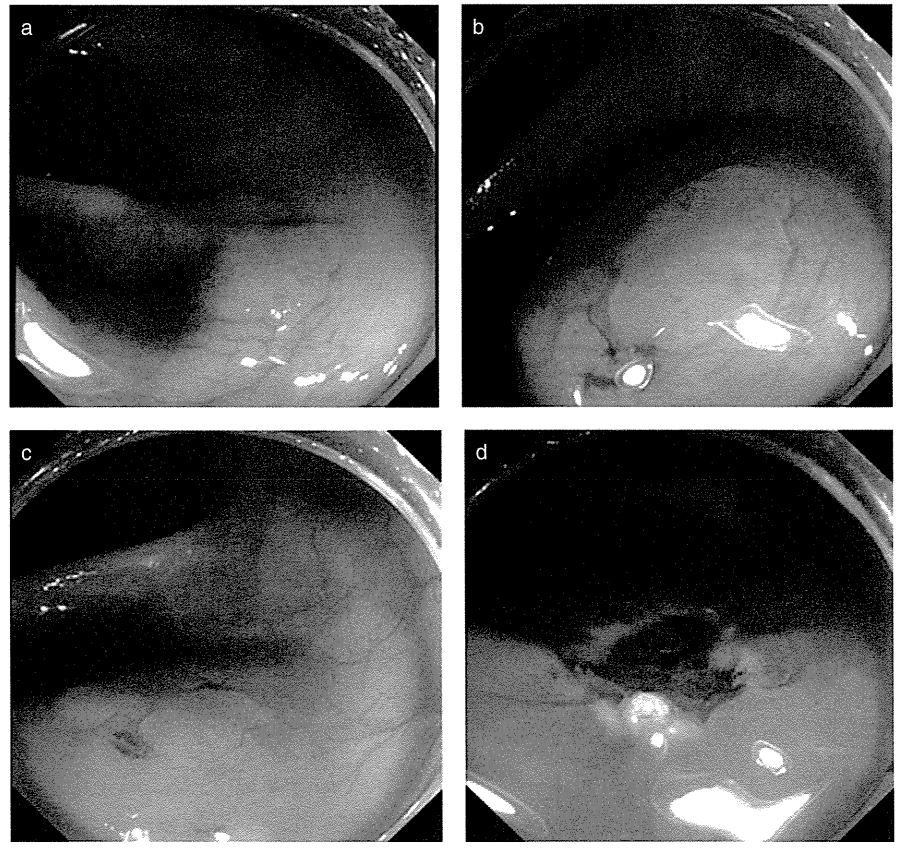

Figure 2 (a) A superficial polyp of 8 mm diameter in the descending colon. (b) Initial high mucosal elevation was achieved after injection of 2.0 mL normal saline (NS). (c) Mucosal elevation was not maintained. (d) The endoscopic mucosal resection (EMR) was performed with a procedure time of 80 s. En bloc and complete resections were not accomplished.

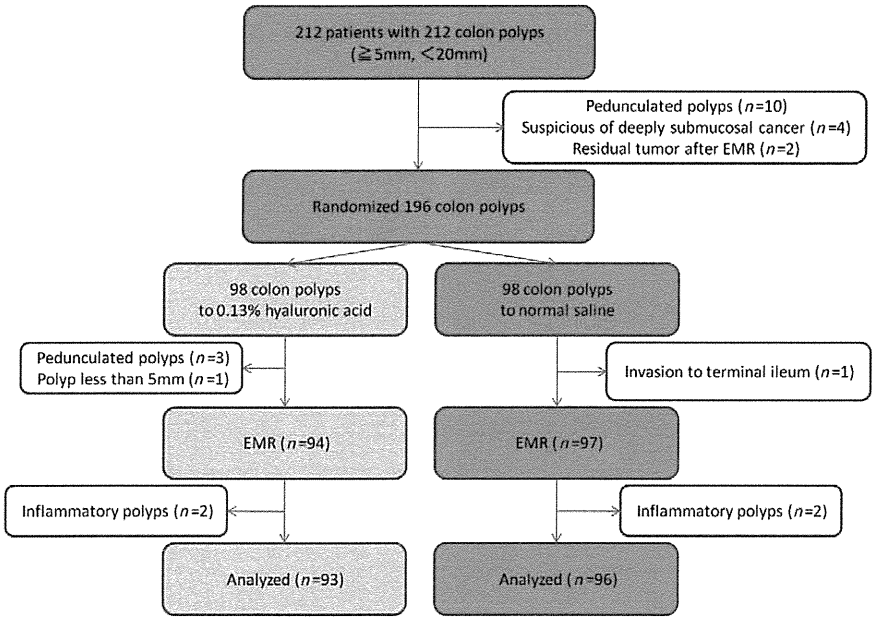

Figure 3 Flow diagram of participant enrollment and randomization classification into the 0.13% hyaluronic acid (HA) group and the normal saline (NS) study arms.

subgroup analysis. Of the seven endoscopists who participated in the study, four were classified as less experienced (having performed less than 2000 colonoscopies) and three as veterans (having performed more than 2000 colonoscopies).

Sample size. We calculated the required sample size on the basis of previous analysis of colorectal EMR to polyps of less than 20 mm diameter in our institution. The rate of complete resection in EMR using NS was about 60%. We hypothesized that 0.13% HA would increase the rate of complete resection by 20% in this study. To assign 80% power for increasing 20% in the rate of complete resection by using a χ^2 test with a 5% significance level in our study, we needed 82 cases per group. We therefore estimated that 100 cases would be required in each group.

Statistical analysis. Statistical analyses were performed using the Mann–Whitney *U*-test and χ^2 test. Continuous variables such as patient age, tumor size, injection volume, and procedure time were analyzed using the Mann–Whitney *U*-test. Categorical variables such as the rate of complete resections and other endpoints were analyzed using the χ^2 test. A *P*-value less than 0.05 was considered statistically significant.

Results

Recruitment of patients and number of colorectal polyps analyzed. A total of 212 patients fulfilled the eligibility criteria for this study; of these 212 patients, 16 were excluded according to our criteria (Fig. 3). Thus, a total of 196 patients were enrolled and randomly assigned into two groups. Seven lesions were excluded according to our criteria. A total of 93 patients in the 0.13% HA group and 96 patients in the NS group were analyzed. The characteristics of both groups are shown in Table 1; moreover, the two groups were not significantly different with respect to various parameters including tumor size, location and morphology. The proportion of sessile polyps and subpedunculated polyps in protruded polyps were not significant in the two groups (18:50 in the 0.13% HA group and 22:55 in the NS group). All superficial polyps in the two groups consisted of elevated type. The ratio of veteran and less-experienced endoscopists administering both the injection solutions was not significantly different.

Table 1 Characteristics of colorectal polyps in the 0.13% hyaluronic acid and normal saline groups

	0.13% hyaluronic acid	Normal saline	
Number of tumors	93	96	
Median age (range)	65.5 (23–85)	66.8 (35–89)	
M/F	62/31	63/33	NS
Tumor size (mm) (range)	8.9 (8–16)	8.2 (5–15)	NS
Location (C to D or S to R)	49: 44	50: 46	NS
Morphology (protruded or superficial)	68: 25	77: 19	NS
Ratio of veteran endoscopists to less experienced endoscopists	0.48 (30/63)	0.52 (33/63)	NS

C, cecum; D, descending colon; NS, not significant; R, rectum; S, sigmoid colon.

Outcomes. Complete resection was achieved in 74 of the 93 lesions (79.5%) in the 0.13% HA group and 63 of the 96 lesions (65.6%) in the NS group (Table 2); the rate of complete resection in the 0.13% HA group was significantly higher than that in the NS group ($P = 0.03 < 0.05$). In the view of the proportions of lateral and vertical margin positive, all 19 cases of incomplete resection in the 0.13% HA group and all 33 cases of incomplete resection in the NS groups were positive of lateral margin. There were no positive of vertical margin in the 0.13% HA group and the NS group.

Table 2 Characteristics of endoscopic mucosal resection (EMR) in the 0.13% hyaluronic acid and normal saline groups

	0.13% hyaluronic acid	Normal saline	
Injection volume (mL)	3.7	3.0	NS
Maintenance of high elevation (%)	83.9%, 78/93	54.1%, 52/96	$P < 0.001$
Average EMR procedure time (s) (range)	150 (55–434)	125 (53–495)	$P < 0.001$
En bloc resection (%)	96.7%, 90/93	97.9%, 94/96	NS
Complete resection (%)	79.5%, 74/93	65.6%, 63/96	$P < 0.05$
Histology Adenoma: Intramucosal cancer	84: 9	92: 4	NS
Perforation (%)	0	0	NS
Postoperative hemorrhage (%)	1.1%, 1/93	1.0%, 1/96	NS

NS, not significant.

Table 3 Subgroup analyses for clinical factors affecting complete resection: morphology, size, location, and endoscopist experience

Morphology	0.13% hyaluronic acid	Normal saline	
Protruded	79.4%, 54/68	62.3%, 48/77	$P < 0.05$
Superficial	80.0%, 20/25	78.9%, 15/19	NS
Polyp size			
5–10 mm	81.6%, 58/71	66.6%, 48/72	$P < 0.05$
11–20 mm	72.7%, 16/22	62.5%, 15/24	NS
Location			
The location: C to D	77.5%, 38/49	68.0%, 34/50	NS
The location S to R	81.8%, 36/44	63.0%, 29/46	NS
Endoscopist experience			
Complete resection by veteran endoscopist	80.0%, 24/30	66.6%, 22/33	NS
Complete resection by less experienced endoscopist	79.3%, 50/63	62.1%, 41/66	$P < 0.05$

C, cecum; D, descending colon; NS, not significant; R, rectum; S, sigmoid colon.

Analysis of the secondary endpoints is shown in Tables 2 and 3. The rate of maintenance of high mucosal elevation in the 0.13% HA group was significantly higher than that in the NS group (83.9% *vs* 54.1%; $P = 0.0001 < 0.001$). Similarly, the average EMR procedure time was significantly longer in the 0.13% HA group than in the NS group (150 s *vs* 125 s; $P = 0.015 < 0.05$). There was no significant difference in the rate of postoperative bleeding between the 0.13% HA group (1.1%) and the NS group (1.0%); moreover, there were no perforations in either of the two groups. En bloc resection was achieved in 90 of 93 lesions (96.7%) in the 0.13% HA group and 94 of 96 lesions (97.9%) in the NS group (N.S.). The diameters of the three lesions without en bloc resection in the 0.13% HA group were 8 mm, 15 mm, and 15 mm. For these three lesions, mucosal elevation was not maintained. The two lesions without en bloc resection in the NS group were 8 mm and 16 mm in diameter, and mucosal elevation was not maintained for one lesion.

The relationship between complete resection and polyp morphology was analyzed (Table 3) and showed a significantly higher complete resection rate of protruded polyps in the 0.13% HA group (79.4%) than in the NS group (62.3%; $P = 0.03 < 0.05$). The relationship between complete resection and polyp size was analyzed and showed a significantly higher complete resection rate of polyps of 5–10 mm diameter in the 0.13% HA group (81.6%) than in the NS group (66.6%; $P = 0.03 < 0.05$). The relationship between the rate of complete resection and the polyp location in both groups was analyzed, and no significant differences were observed between the two groups with respect to the location. The relationship between complete resection and the experience of the endoscopist was also analyzed. The rate of complete resection by less-experienced endoscopists was significantly higher in the 0.13% HA group (79.3%) than in the NS group (62.1%; $P = 0.03 < 0.05$).

Discussion

The efficacy of HA in clinical colorectal EMR has been reported previously.[17,22] Hirasaki *et al.* reported a prospective multicenter open-label study showing the utility of 0.4% HA in colorectal EMR of 40 colon polyps of 5–20 mm in diameter.[17] The rate of complete resection was high (82.5%, 33/40) and mucosal lesion-lifting was adequate (75.0%, 30/40). Here, we observed similar results by using 0.13% HA in the rates of complete resection (79.5%) and maintenance of high elevation (83.9%). The rates of complete resection and maintenance of high elevation in the NS group in our study were not high, i.e. 65.6% and 54.1%, respectively, suggesting that the maintenance of high elevation by 0.13% HA improved the complete resection rate. Hurlstone *et al.* also reported the use of HA in a randomized endoscopist-blinded study of colorectal EMR.[22] However, they did not report the concentration of HA used; they compared HA with dextrose and analyzed colorectal polyps of < 30 mm diameter. Complete resection was achieved in 56 of the 81 lesions (69%). It is possible that this rate is lower than that observed in our study because the median size of the polyps (20.2 mm) was larger than that of the polyps included in our study (8.9 mm). We suspected that the rate of complete

resection with EMR using HA was high; however, the efficacy of HA was confirmed for the polyps of < 20 mm diameter. The efficacy of EMR using HA for polyps > 20 mm diameter should be evaluated in further studies. On the other hand, it is known that most cases with en bloc resection does not have residual tumor even if they have tumor-positive margins because of coagulation artifacts in resected lateral margin of specimens. However, more follow-up colonoscopy is performed when the lateral positive margin is detected in the case of intramucosal cancer, because recurrence of the tumor occurs in a part of cases with tumor-positive margins. Moreover, definite pathological diagnosis whether the tumor is adenoma or cancer is sometimes disturbed by coagulation artifacts in resected specimens. In view of these points, it is important to achieve negative lateral margin in all polyps for definite histopathological diagnosis and prevention of unnecessary follow-up colonoscopy and recurrence of the tumor.

The rate of postoperative hemorrhage in the two groups in our study was not significantly different (1.1% in the 0.13% HA group and 1.0% in the NS group). In a recent report on EMR in Japan, the rate of postoperative hemorrhage was approximately 1.2% ($n = 36\,083$ lesions), which is similar to our data.[23] However, another study showed that the rate of postoperative hemorrhage in EMR using HA was 4.9%, with 3.7% occurring within 24 h and 1.2% after 24 h.[22] Another study reported a postoperative hemorrhage rate of 4.9% for EMR using HA.[17] Collectively, these studies suggest that the rate of postoperative hemorrhage in EMR using HA is higher than that with NS. However, our data did not show this. To address this, we have recently designed a multicenter trial to evaluate the rate of postoperative hemorrhage in EMR using HA; this study is currently in progress.

In our study, there was no perforation in either group. Two other studies with HA reported perforation rates of 0% and 1.2%.[17,22] The large study of EMR in Japan reported an average perforation rate of 0.9% for EMR ($n = 36\,083$ lesions).[23] Theoretically, maintenance of high mucosal elevation by using HA can prevent perforation. We expect that this will be clarified in our large-scale multicenter trial of EMR with 0.13% HA.

In the current study, the average EMR procedure time in the 0.13% HA group (150 s) was longer than that in the NS group (125 s). This was likely due to the higher injection pressure required for 0.13% HA than for NS.[9] According to the average EMR procedure time in both the groups, a minimum of 2 min maintenance of mucosal elevation should be expected after mucosal injection. Our previous report demonstrated that HA concentrations higher than 0.13% could maintain mucosal elevation for more than 2 min in the resected porcine colon, esophagus, and living minipig colon.[9] However, dilution of HA was necessary because the viscosity of high concentrations of HA can make snaring difficult and increase the injection pressure. Moreover, HA is more expensive than NS. It cost 7700 yen for a 20 mL-bottle of 0.4% HA in Japan, compared to 61 yen for a 20 mL-bottle of NS. Dilution of HA is also important in view of decreasing cost.

Our study analyzed the relationship between complete resection and morphology of the polyps. The rate of complete resection of protruded polyps in the 0.13% HA group (79.4%) was significantly higher than that in the NS group (62.3%). Subpedunculated lesion may be easily resected by EMR due to small area of adhesion to colonic wall in comparison with its maximum diameter. However, the proportions of sessile polyps and subpedunculated polyps were not significant in the two groups. There was no significant difference in the complete resection rate of superficial polyps in the two groups. The reason for this is unclear because there was no difference between the groups with respect to the rate of maintenance of high elevation in superficial polyps (76.0% in the 0.13% HA group and 73.7% in the NS group). Moreover, there were no significant difference about median size of superficial polyps between two groups (11.2 mm in the 0.13% HA group and 10.3 mm in the NS group). One possible reason was that 0.13% HA solution was harder than NS and it might cause the necessity of pressing of superficial polyp for complete resection. Less experienced endoscopists might not do that in the early period. It is possible that 0.13% HA might not be useful to snare superficial polyps or there may have been very few cases to demonstrate significance in this study. A relationship between complete resection and the experience of the endoscopist was demonstrated in our study. The rate of complete resection of polyps by less-experienced endoscopists was significantly higher in the 0.13% HA group (79.3%) than that in the NS group (62.1%). One reason for this might have been that high mucosal elevation by 0.13% HA that facilitated snaring. The EMR procedure time of less-experienced endoscopists was longer than that of the veteran endoscopists because of the learning curve. We suggest that less-experienced endoscopists should use 0.13% HA in EMR to prevent perforation, which can be caused by low mucosal elevation. We expect that the multivariate analyses for the complete resection including parameters of tumor size, morphology, type of injection solution, degree of mucosal elevation and endoscopists experience will be performed in our large-scale multicenter trial.

In conclusion, we have shown that EMR to colorectal polyps of < 20 mm diameter using 0.13% HA was effective for high rates of histopathologically confirmed complete resection, especially for protruded polyps. In addition, 0.13% HA maintained high mucosal elevation and was associated with better complete resection rates by less-experienced endoscopists.

Limitations. The evaluation of high mucosal elevation could be subjective though it was judged by the agreement of evaluation by two endoscopists. There is a possibility that bias was introduced, because only seven endoscopists performed EMR in this study. However, the ratio of veteran to less-experienced endoscopists for the HA and NS injection solutions was not significantly different. No follow-up colonoscopy was performed to assess the site for recurrence.

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Recognition of Endoscopic Diagnosis in Differentiated-Type Early Gastric Cancer by Flexible Spectral Imaging Color Enhancement with Indigo Carmine

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Key Words
Differentiated-type early gastric cancer · Early gastric cancer · EGC demarcations · Endoscopic diagnosis · Endoscopic submucosal dissection · Flexible spectral imaging color enhancement

Abstract
Background/Aims: To evaluate the usefulness of flexible spectral imaging color enhancement with indigo carmine (I-FICE) in early gastric cancer (EGC) demarcation. ***Methods:*** The study participants were 29 patients with differentiated-type EGC. The endoscope was fixed and images of the same area of EGC demarcations in each lesion were obtained using four different methods (WLE, flexible spectral imaging color enhancement (FICE), CE, and I-FICE). FICE mode at R 550 nm (Gain: 2), G 500 nm (Gain: 4), and B 470 nm (Gain: 4) was used. Four endoscopists ranked the images obtained by each method on the basis of the ease of recognition of demarcation using a 4-point system. We calculated the standard deviation of pixel values based on L*, a*, and b* color spaces in the demarcation region (Lab-SD score). ***Results:*** The median ranking score for I-FICE images was significantly higher than that obtained from the other methods. Further, the average Lab-SD score was significantly higher for I-FICE images than for images obtained by the other methods. There was a good correlation between the ranking score and Lab-SD score. ***Conclusion:*** EGC demarcations were most easily recognized both subjectively and objectively using I-FICE image, followed by CE, FICE and WLE images.

Introduction

Gastric cancer is the second most common cause of cancer-associated deaths worldwide [1]. In Japan, the 5-year survival rate of patients with early gastric cancer (EGC) has been reported to be over 90% following gastrectomy with complete removal of primary and secondary lymph nodes [2, 3]. Therefore, early diagnosis and treatment are the keys to improving the survival rate in cases with gastric cancer.

There have been considerable technical advances in the endoscopic treatment of EGC since the introduction of endoscopic submucosal dissection (ESD). ESD was developed to improve the rate of en-bloc resection for EGC [4, 5]. Although it is necessary to obtain a clear diagnosis

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of EGC demarcation for proper treatment, it is frequently difficult to obtain a clear demarcation.

Chromoendoscopy (CE) came into clinical use 40 years ago as a method of identifying and observing lesions in detail [6]. Indigo carmine is the most frequently used dye for CE in Japan. CE with this dye is more successful than conventional endoscopy for identifying lesion demarcation [7, 8].

Flexible spectral imaging color enhancement (FICE) is a computerized virtual CE imaging tool that was recently invented for use in image-enhanced endoscopy [9]. This tool is being further developed for diagnosing gastrointestinal lesions [10–16]. In FICE, an ordinary endoscopic image is obtained from the video processor and the reflected photons are arithmetically processed to reconstruct virtual images for a selection of different wavelengths. FICE is designed to enhance visualization of the vasculature network and surface structure of the mucosa to obtain improved tissue characterization, differentiation, and diagnosis. This technique is considered as a potential alternative to CE because it provides contrast enhancement of the tissue surface structures; however, FICE is yet to be extensively studied as a CE tool. In this study, we investigated the usefulness of FICE and FICE of endoscopy with indigo carmine (I-FICE) for the recognition of EGC demarcation as compared to conventional white light endoscopy (WLE) and CE.

Patients and Methods

Patients

Between April 2009 and May 2010, 29 consecutive patients with EGC who underwent ESD at the University Hospital, Kyoto Prefectural University of Medicine, were enrolled in this study. In all these cases, differentiated adenocarcinoma was diagnosed from the biopsy specimen for histopathology before ESD. All the patients provided written informed consents for undergoing gastroendoscopic examinations, including FICE and I-FICE, and the study was approved by the ethics committee of Kyoto Prefectural University of Medicine.

Gastroendoscopy

All the procedures were carried out with an EG-590ZW endoscope and an Advancia endoscopic system (Fujifilm Co., Tokyo, Japan). The FICE can be applied with the push of a button. FICE was used at R 550 nm (Gain: 2), G 500 nm (Gain: 4), and B 470 nm (Gain: 4). The same endoscopy system settings were used for all four methods – WLE, FICE, CE, and I-FICE. The endoscope was fixed at one angle for observing the lesions, and each lesion at that fixed angle was recorded using WLE, FICE, CE, and I-FICE. First, the lesion was visualized using WLE, and the image was saved. The mode was then changed to FICE to capture the FICE image of the lesion. For CE, 10–20 ml of 0.2% indigo carmine dye was placed on the lesion, and a CE image was then obtained. Finally, I-FICE of the CE image was performed. All the images were captured and recorded in a digital filing system. This study aimed to evaluate the quality of the still images obtained using these four methods and to compare their efficacy of lesion visualization and assessment.

Histopathology

ESD was performed on all 29 lesions, which were then extended on boards with pins for fixation in 20% formalin. Each lesion, together with the surrounding mucosa, was cut into 2-mm wide serial-step sections. Histopathological criteria for diagnosis of EGC demarcations were based on the Japanese Classification of Gastric Carcinomas [17].

Sensory Evaluation

We scored the evaluation of the endoscopic images using the following 4-point ranking method based on ease of recognition of lesion demarcation. Images with the easiest recognition were given 4 points; those with comparatively lower degree of clarity were given 3; those that were very obscure were given 2, and the most obscure images scored only 1 point.

The four endoscopic images (WLE, CE, FICE, and I-FICE images) of each lesion were prepared for evaluation by placing on a slide. Each image was scored from a scale of 1–4 points; the points were assigned according to the ease of recognition of lesion demarcation. The images were rated by four endoscopists (T.W., N.Y, N.B., and S.Y.), who have previously evaluated over 100 conventional WLE and FICE images prior to this study. The endoscopists were blinded to each other's scores. None of the observers had previously seen any of the endoscopic images in this study. The scores were referred to as the 'ranking scores'. The median ranking score for each method was determined by calculating the median score for the 29 lesion images scored by four different endoscopists.

In an independent experiment, we examined the inter- and intraobserver variabilities while assigning the score. All the four endoscopists scored and ranked the images of each case in the order of presentation in a day. One week later, the same images were presented in a different order for scoring and ranking by the same endoscopists. Each endoscopist drew a line recognized as a demarcation line on the 4-point acquired image of each of the 29 lesions. Demarcations identified during endoscopic imaging were compared with those identified histopathologically from resected specimens.

Objective Evaluation

The following methods were used to ensure that the endoscopic images were objectively evaluated. The area of each lesion containing the demarcation was captured for image processing, and the region of interest (ROI) was highlighted. In order to ensure the accuracy of this method, the ROIs were selected under the following conditions: (1) ROIs were selected at the same position in all four images of a particular lesion; (2) ROIs were selected so that the areas other than EGC and EGC may become in halves, and (3) domains with excess brightness or darkness or with particular halations were excluded.

Standard deviation (SD) of the pixel values within the ROI was used to evaluate the contrast of each color image. Each image was assigned three pixel values (L*, a*, and b*) in the CIELAB color

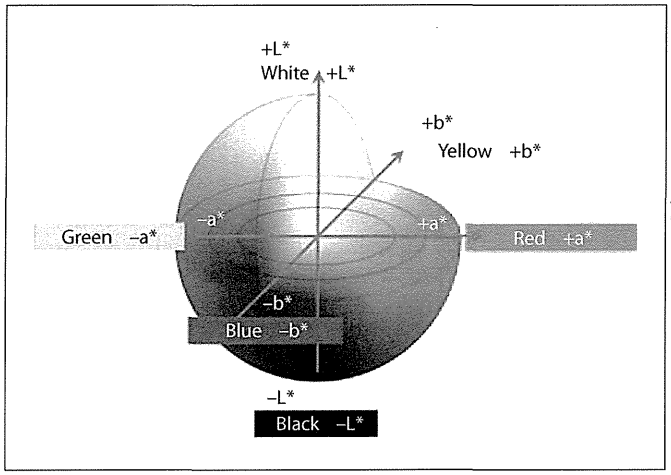

Fig. 1. In the CIELAB color space system the color differences are visualized as distances in a diagram. L*: color brightness (L* = 0 is black and L* = 100 is white). a*: position between red and green (negative values are pro-green, positive values are pro-red). b*: position between yellow and blue (negative values are pro-blue, positive values are pro-yellow).

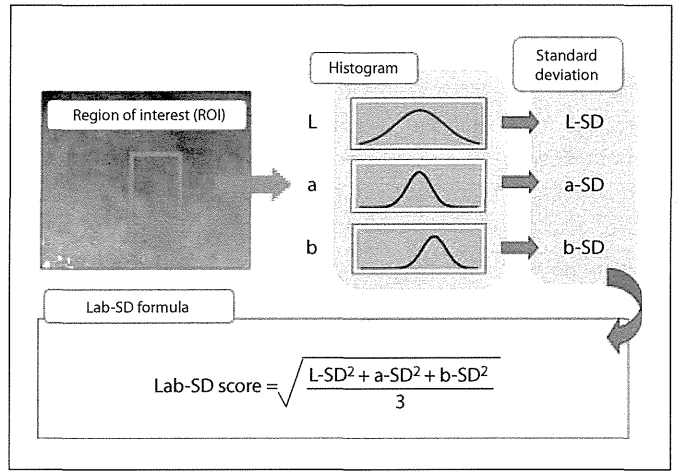

Fig. 2. Lab-SD method. The RMS the pixel values with SDs based on L*, a*, and b* color spaces (Lab) for each image (Lab-SD score) was calculated to obtain the total contrast score.

space system (fig. 1); three SDs were calculated on the basis of these values. The CIE 1976 (L*, a*, and b*) color space (CIELAB) was used as the color-opponent space specified by Commission Internationale de l'Eclairage (CIE) with the dimensions L for lightness, and a and b for color-opponent dimensions. This color space is more perceptually uniform than other color spaces, in other words the amount of change in a color value would produce a similar change in visual importance [18]. The root mean square (RMS) value of the three SDs was calculated as the total contrast score. The average RMS score was calculated for each method. We termed this method 'Lab-SD', and the resultant RMS score was the Lab-SD score (fig. 2). The Spearman rank correlation coefficient was used to determine the rank correlation of the ranking score and the Lab-SD score in each EGC.

Statistical Analysis

The median ranking scores for the four methods were compared using Mann-Whitney U test to determine any significant differences among the imaging methods. A p value <0.05 was considered statistically significant. The inter- and intraobserver variability values were quantified by the κ statistic, which measures agreement over and above chance agreement. κ values of <0.4, 0.4–0.75, and >0.75 were considered to indicate poor, fair-to-good, and excellent agreement, respectively. The average Lab-SD scores for the four methods were compared using Student's t test. A p value <0.05 was considered statistically significant. The Spearman correlation coefficient can take on any value from 0 to 1, where higher value means higher agreement. Spearman correlation coefficients of <0.5, 0.5–0.9, and >0.9 were considered to indicate poor, fair-to-good, and excellent agreement, respectively. All the statistical analyses were performed using SPSS 15.0 software (SPSS Inc., Chicago, Ill., USA).

Table 1. Clinicopathological features of patients with EGCs

Features	n
Median age, years (range)	61 (50–79)
Gender	
Male	27
Female	2
Mean tumor size, mm (range)	14.5 (2–30)
Histological type	
Well differentiated	27
Moderately differentiated	2
Depth	
Intramucosa	23
Submucosa	6
Macroscopic type	
Elevated type	14
Depressive type	15

Results

Histopathological Findings

Resected lesions were 27 well-differentiated and 2 moderately-differentiated adenocarcinomas with carcinoma-free lateral margins. The clinicopathological features of the evaluated lesions are summarized in table 1.

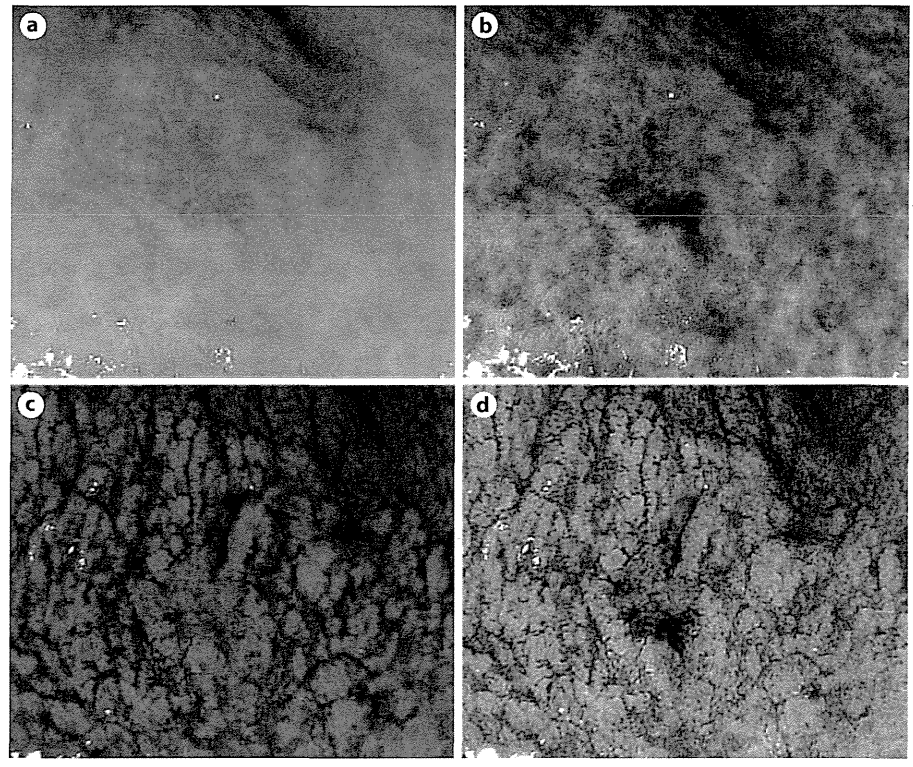

Fig. 3. Representative sets of still images; case 1. Superficial depressive lesion (0–IIc) on the lesser curvature of the middle gastric body. **a** WLE image. **b** FICE image. **c** CE image. **d** I-FICE image.

Representative Cases

Case 1 is a superficial depressive lesion (0–IIc) on the lesser curvature of the middle gastric body (fig. 3). A flat lesion is visible on the lesser curvature of the middle gastric body, but the demarcation of the lesion is unclear in WLE image (fig. 3a). The demarcation of the lesion is more clearly distinguishable because of the reddish color of the tumor in FICE image (fig. 3b). The irregular mucosal structure of the tumor can be visible and the tumor can be recognized superficial depressive lesion in CE image (fig. 3c). The mucosal structure as well as color of the tumor can also be more clearly visualized in I-FICE image (fig. 3d). The resected lesion by ESD was diagnosed an intramucosal well-differentiated adenocarcinoma with carcinoma-free lateral margins histopathologically (fig. 4a–c). One of four endoscopists (T.W.) chose I-FICE image as a 4-point acquired image and drew the line as a demarcation line on the I-FICE image of the lesion (fig. 4d). The mapping transformations of the each endoscopic image were determined by the mapping of each resected specimen (fig. 4e). The demarcation line using an endoscopic image matches the histopathological mapping of each resected specimen (fig. 4f).

Case 2 is a superficial elevated lesion (0–IIa) on the lesser curvature of the upper gastric body (fig. 5). A flat-elevated lesion is visible on the lesser curvature of the upper gastric body, but the demarcation of the lesion is partially unclear in WLE image (fig. 5a). The demarcation of the lesion is more clearly distinguishable because of the white color of the tumor in FICE image (fig. 5b). The mucosal structure of the tumor is more visible in CE image (fig. 5c). The mucosal structure as well as color of the tumor can also be clearly visualized in I-FICE image (fig. 5d). The resected lesion by ESD was diagnosed an intramucosal well-differentiated adenocarcinoma with carcinoma-free lateral margins histopathologically (fig. 6a–c). One of four endoscopists (N.Y.) chose I-FICE image as a 4-point acquired image and draw the line as a demarcation line on the I-FICE image of the lesion (fig. 6d). The mapping transformations of the each endoscopic image were determined by the mapping of each resected specimen (fig. 6e). The demarcation line using endoscopic image matches the histopathological mapping of each resected specimen (fig. 6f).

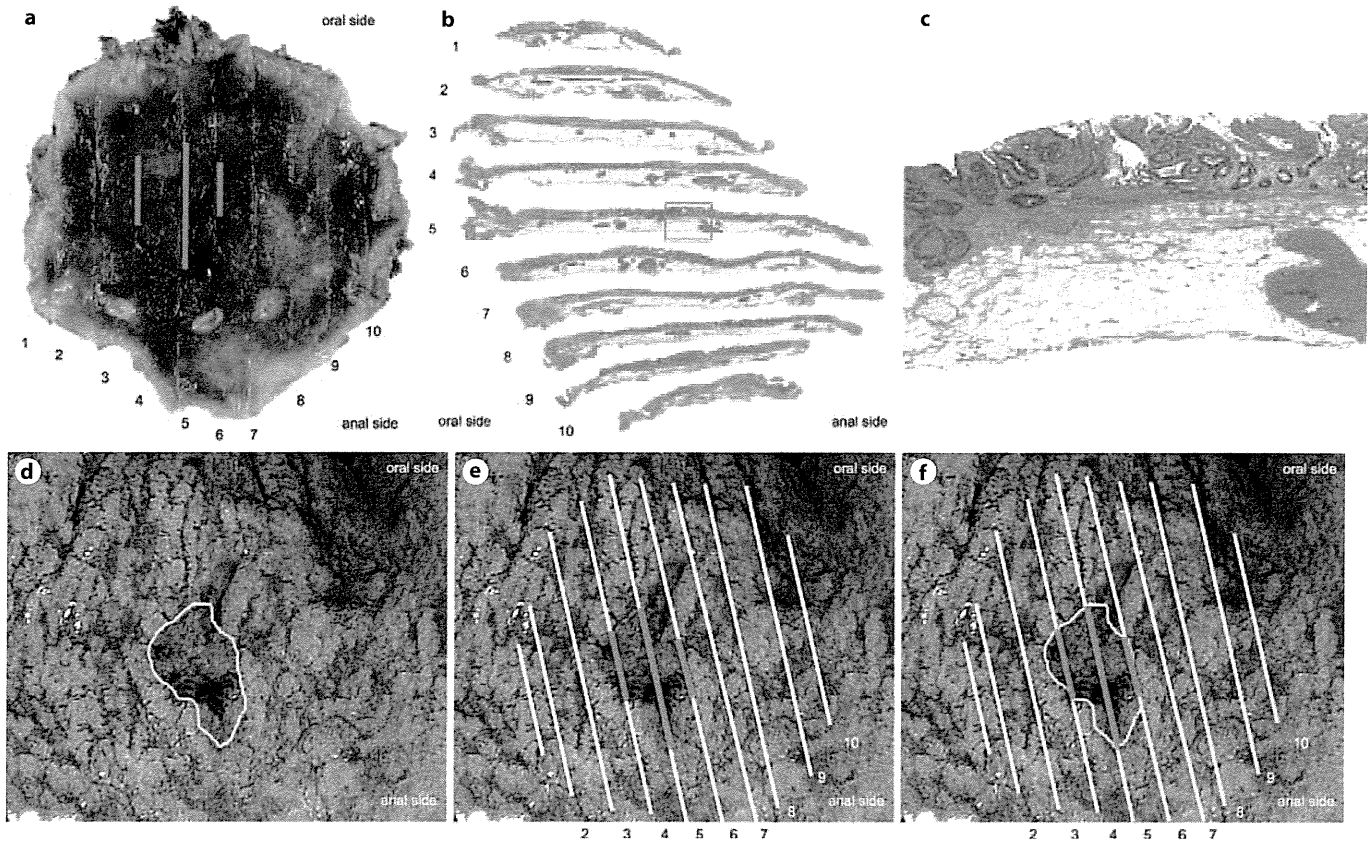

Fig. 4. Histopathlogical and endoscopic mapping; case 1. **a** The mapping of a resected specimen. **b** Loupe images of sections in the resected specimen. **c** Low magnification of boundary region between cancer and non-cancerous mucosa in the section No. 5 (**b**, red square). **d** The yellow line was drawn as a demarcation line of the lesion on I-FICE image. **e** The mapping transformations of the I-FICE image were determined by the mapping of a resected specimen. **f** The yellow line demarcation identified using endoscopic image was verified the mapping histopathologically. Adenocarcinoma exists on red lines in the mapping of each figure. Non-cancerous region exists on white lines in the mapping of each figure. Arabic numerals represent the number of sections. The oral side and the anal side are represented on the each figure, respectively.

Ranking Score

The median ranking score for each method was as follows: WLE, 1.03 ± 0.16; FICE, 2.25 ± 0.59; CE, 2.84 ± 0.52, and I-FICE, 3.87 ± 0.33. According to the Mann-Whitney U test, the differences between the median ranking values among the four different imaging methods were statistically significant ($p < 0.001$). Moreover, the median ranking score of I-FICE was found to be significantly higher than the scores obtained by the other three methods, and both FICE and CE scored significantly higher than WLE (fig. 7).

We determined the κ values for interobserver (T.W., N.Y., N.B., and S.Y.) variability in the evaluation of images obtained by the four methods. The κ values of interobserver variability for a 4-point acquired image, which is the easiest to recognize, were as follows: T.W. to N.Y. was 0.642; T.W. to N.B., 0.642; T.W. to S.Y., 0.787; N.Y. to N.B., 0.874; N.Y. to S.Y., 0.843, and N.B. to S.Y., 0.724. The κ values of intraobserver variability between the four endoscopists for the images rated 4 were 0.642 (T.W.), 0.721 (N.Y.), 0.622 (N.B.), and 0.820 (S.Y.) (table 2).

All of four endoscopists compared with the demarcation lines using the 4-point acquired image and pathological demarcations in the 29 lesions. All 29 demarcations identified using a 4-point acquired image were verified histopathologically among four endoscopists.

Lab-SD Scores

Representative image sets are shown in figure 3. We determined a contrast score for each image in the captured area of the lesion containing the demarcation (fig. 8). The average Lab-SD score for each imaging meth-

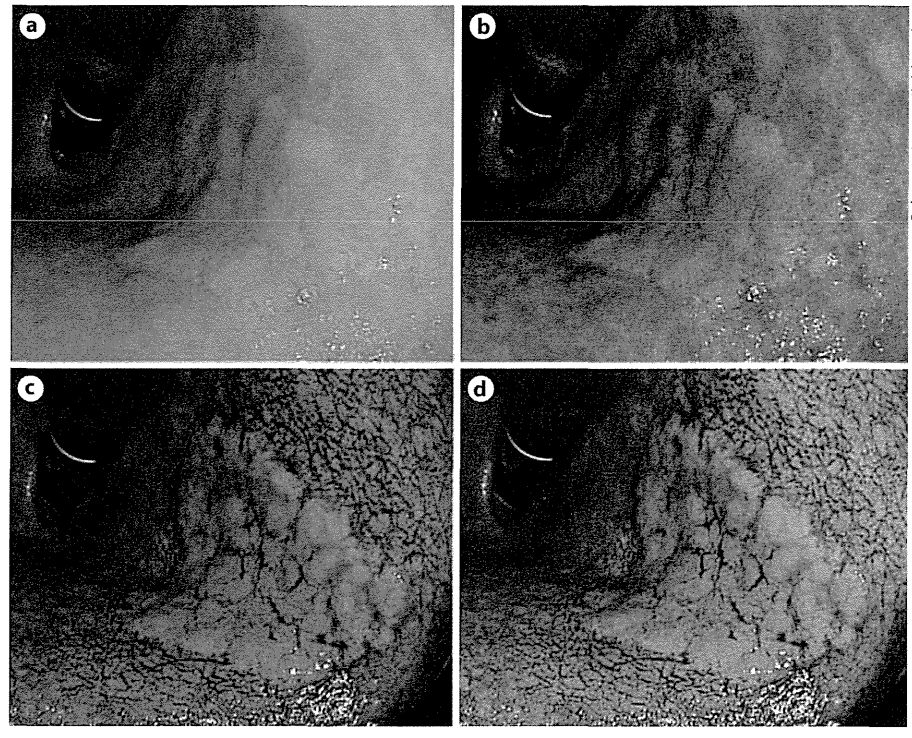

Fig. 5. Representative sets of still images; case 2. Superficial elevated lesion (0–IIa) on the lesser curvature of the upper gastric body. **a** WLE image. **b** FICE image. **c** CE image. **d** I-FICE image.

Table 2. Inter- and intraobserver variability for 4-point acquired image (κ value)

	T.W. to N.Y.	T.W. to N.B.	T.W. to S.Y.	N.Y. to N.B.	N.Y. to S.Y.	N.B. to S.Y.
Interobserver variability	0.642	0.642	0.787	0.874	0.843	0.724
	T.W.	N.Y.	N.B.	S.Y.		
Intraobserver variability	0.642	0.721	0.622	0.820		

od were as follows: WLE, 5.38 ± 1.30; FICE, 7.52 ± 1.74; CE, 8.98 ± 2.46, and I-FICE, 10.4 ± 2.20 (fig. 9). There were significant differences between the average Lab-SD scores obtained using the four different imaging methods, and the average Lab-SD score for I-FICE images was significantly higher than that obtained from the other methods ($p < 0.001$). The image rating and Lab-SD score were correlated, and I-FICE showed the highest score, followed by CE, FICE, and WLE.

Further, we calculated the Spearman rank correlation coefficients between the image rating and Lab-SD score in each image from four endoscopists (T.W., N.Y., N.B., and S.Y.), and the values were 0.934 (T.W.), 0.959 (N.Y.), 0.948 (N.B.), and 0.936 (S.Y.).

Discussion

This is the first report demonstrating the usefulness of I-FICE for the recognition of EGC demarcation in comparison to FICE, CE, and WLE imaging. The recently developed FICE system enhances the mucosal surface and microvessel architecture in the images; several studies have suggested the comparable diagnostic accuracies of FICE and CE in digestive lesions such as Barrett's esophagus [10, 11], EGC [12–14], and colorectal polyps [15, 16]. In the present study, the FICE mode was used at R 550 nm, G 500 nm, and B 470 nm wavelengths, on the basis of the findings from previous studies. Previous reports have indicated that a special set of wavelengths is consid-

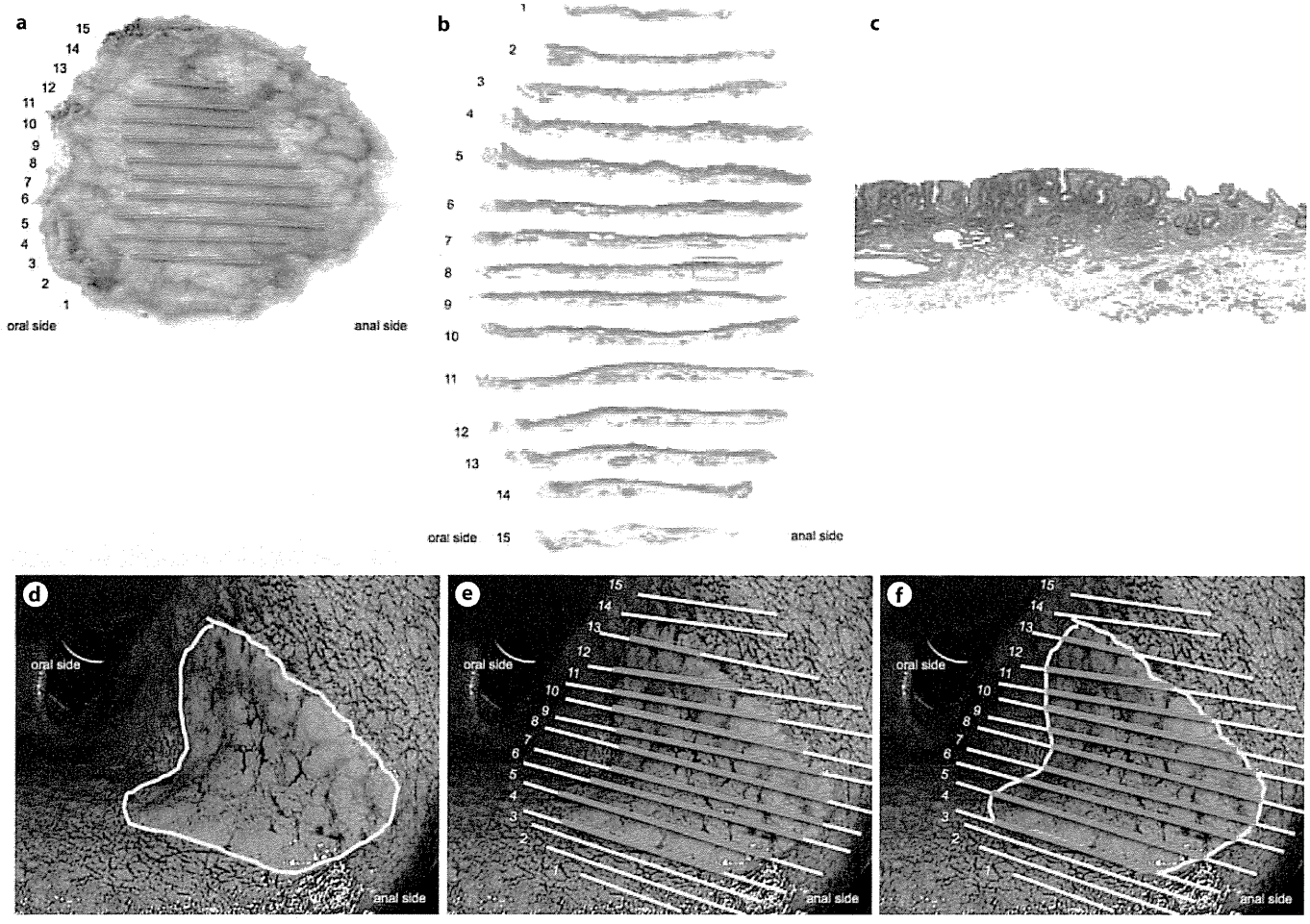

Fig. 6. Histopathlogical and endoscopic mapping; case 2. **a** The mapping of a resected specimen. **b** Loupe images of sections in the resected specimen. **c** Low magnification of boundary region between cancer and non-cancerous mucosa in the section No. 8 (**b**, red square). **d** The yellow line was drawn as a demarcation line of the lesion on I-FICE image. **e** The mapping transformations of the I-FICE image were determined by the mapping of a resected specimen. **f** The yellow line demarcation identified using endoscopic image was verified the mapping histopathologically. Adenocarcinoma exists on red lines in the mapping of each figure. Non-cancerous region exists on white lines in the mapping of each figure. Arabic numerals represent the number of sections. The oral side and the anal side are represented on the each figure, respectively.

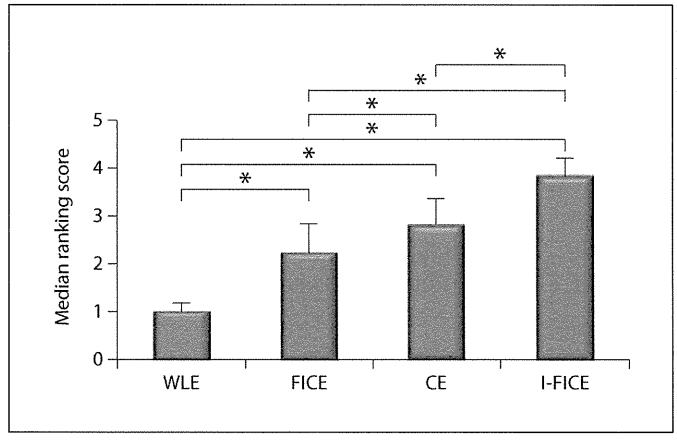

Fig. 7. Ranking score of four methods by endoscopists. The median score (with SD) obtained using each of the four methods evaluated by 4 separate endoscopists. WLE, conventional white-light endoscopy; FICE, flexible spectral imaging color enhancement endoscopy; CE, conventional white light endoscopy with indigo carmine; I-FICE, FICE of endoscopy with indigo carmine. * $p < 0.05$ by Mann-Whitney U test.

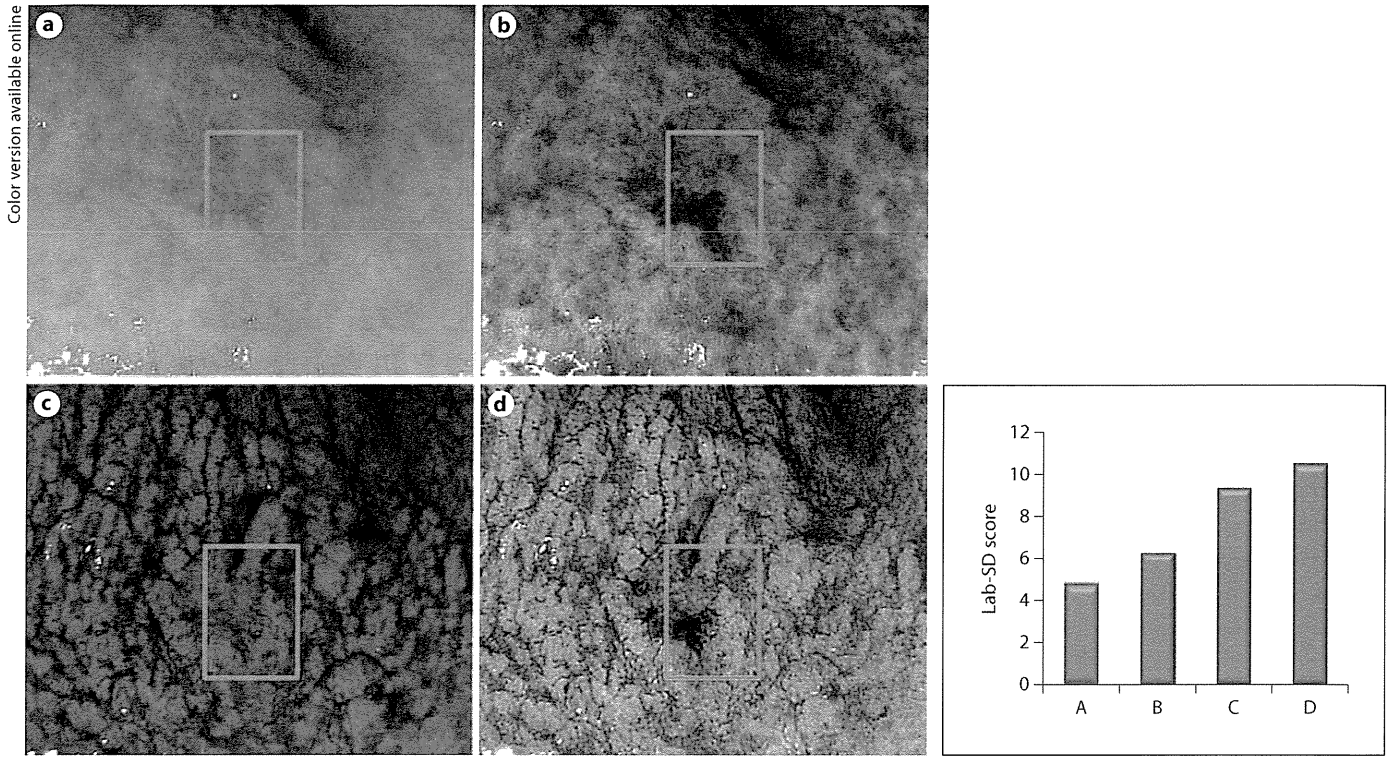

Fig. 8. Representative sets of images illustrating the lesion area containing the demarcation (fig. 3). **a** WLE image, **b** FICE image, **c** CE image, **d** I-FICE image. The squares represent the ROI of each lesion containing the demarcation for calculating the Lab-SD score of the four methods. The bar graph presents the average Lab-SD score obtained by using each of these methods.

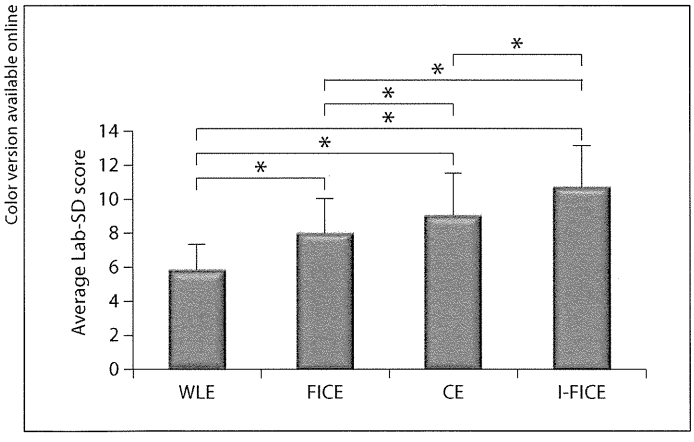

Fig. 9. Average Lab-SD score. The average Lab-SD score (±SD) of each of the four methods. WLE, conventional white light endoscopy; FICE, flexible spectral imaging color enhancement endoscopy; CE, conventional white light endoscopy with indigo carmine; I-FICE, FICE endoscopy with indigo carmine. * $p < 0.05$ with the Student's t test.

erably useful in the detection of elevated and depressed-type EGCs, and that it can be used to enhance the microstructural and microvascular patterns of the cancer surface [12, 13]. The combination of FICE with indigo carmine accentuated the unique appearance of the surface structures and capillary vessels of EGCs, resulting in an increase in the ranking scores. In our study, FICE images showed clearer demarcation than the corresponding WLE images, however the FICE image alone did not prove to be better than the CE image for the recognition of lesion demarcation. These results suggest that FICE is superior to WLE with respect to color enhancement but is inferior to CE with respect to surface structure enhancement. However, I-FICE images exhibited the cumulative beneficial results of CE and FICE as I-FICE facilitated easier demarcation of tumors as compared to CE or FICE alone. The κ value of interobserver variability for images given 4 points from four observers was >0.40. Moreover, the κ value for intraobserver variability for images given 4 points was also >0.40. Therefore, the image evaluation performed using the rating system in this

study was accurate because these values demonstrated fair-to-good or excellent agreement.

In a previous study on the colorimetric evaluation of WLE and FICE images, Osawa et al. [11] examined color difference using CIELAB with transnasal endoscopy between the palisade vessels and background Barrett's esophagus. They selected 5 random sample points in the palisade vessels and background Barrett's esophagus, and calculated the color difference between two objects by using the sample points on WLE and FICE images. The color difference in the FICE images was found to be significantly higher than that in the WLE images ($p < 0.0001$), suggesting that the palisade vessels were clearly recognizable using the FICE system. On the other hand, it would be more difficult to select sample points for the EGC demarcation, such as in this study, because of the lack of uniformity in structure and color in the EGC and tumor surroundings, which are usually present around EGC, and non-uniformities such as inflammation and atrophic changes in the tissue. Therefore, rather than sample points, we selected ROIs containing EGC demarcations in each image in this study in order to overcome these difficulties.

Moreover, we used Lab-SD as an index of contrast recognition of the ROI image. In previous studies, the usefulness and generality of SD of the luminance as a measure of contrast has been confirmed [19]. This is the first report where Lab-SD was used as an index of recognition for EGC demarcation. Spearman rank correlation coefficients revealed that the Lab-SD scores, which objectively evaluated the contrast in the ROI, have a good correlation with the ranking scores, which is a subjective measure of the images by endoscopists. This suggests that Lab-SD is useful as an index of recognition for determining EGC demarcation. However, it also poses a limitation: images containing large areas of halation or darkness in the ROI might create problems in evaluation. In such cases, the contrast of the ROI is greatly affected by the color of halation. Therefore, we selected ROIs excluding domains that were too dark or bright or with halation.

Our study has two limitations. First, the number of patients enrolled in this study was small. However, the statistical difference was significant substantially. Second, we investigated the ease of recognition of differentiated adenocarcinoma demarcation in this study. Undifferentiated adenocarcinoma was not included in the present study, because undifferentiated adenocarcinoma is often difficult to be identified the demarcation in endoscopic diagnosis. So, further studies are required for the endoscopic diagnosis of the demarcation of undifferentiated adenocarcinoma.

In our study, the average Lab-SD score of I-FICE images was significantly higher than that obtained by the other three methods, and both the ranking score and Lab-SD score were in agreement. Therefore, among the four imaging methods trialed in this study, I-FICE is the best imaging tool in the recognition of EGC demarcation, both subjectively and objectively. Currently, FICE has a limitation in structure enhancement as compared with CE. In the near future, the FICE technique should be developed to enhance contrast without requiring endoscopy with indigo carmine.

Conclusion

In conclusion, the EGC demarcations were recognized and diagnosed most easily both subjectively and objectively in I-FICE images; further, FICE and CE images had better clarity than WLE. These results suggest that the performance of I-FICE is better because it exhibited the cumulative benefits of CE and FICE. This new method is a promising diagnostic approach for improved assessment of EGC demarcations and for the early diagnosis of EGC.

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RESEARCH ARTICLE

Phase II trial of combined regional hyperthermia and gemcitabine for locally advanced or metastatic pancreatic cancer

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Abstract

Purpose: Despite advances in cancer therapy, treating pancreatic cancer remains one of the major challenges in the field of medical oncology. We conducted this phase II study to evaluate the efficacy and safety of regional hyperthermia combined with gemcitabine for the treatment of unresectable advanced pancreatic cancer.

Methods: Eligibility criteria included histologically proven, locally advanced or metastatic pancreatic cancer. Gemcitabine was administered intravenously at a dose of 1000 mg/m^2 on days 1, 8, and 15 every 4 weeks. Regional hyperthermia was performed once weekly, 1 day preceding or following gemcitabine administration. The primary end point was the 1-year survival rate. Secondary objectives were determination of tumour response and safety.

Results: We enrolled 18 patients with advanced pancreatic cancer between November 2008 and May 2010. The major grade 3–4 adverse events were neutropenia and anaemia; however, there were no episodes of infection. The objective response rate (ORR) and disease control rate (ORR + stable disease) were 11.1% and 61.1%, respectively. Median overall survival (OS) was 8 months, and the 1-year survival rate was 33.3%. Median OS of patients with locally advanced pancreatic cancer was 17.7 months.

Conclusions: Regional hyperthermia combined with gemcitabine is well tolerated and active in patients with locally advanced pancreatic cancer.

Keywords: *advanced pancreatic cancer, gemcitabine, hyperthermia, phase II study*

Introduction

Pancreatic ductal adenocarcinoma is the fifth most common cause of cancer-related death in Japan. Although tumour resection is the only curative treatment, approximately 80% of patients are ineligible for surgery because of unfavourable tumour location and metastatic disease. Gemcitabine became the standard chemotherapeutic agent for

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locally advanced and metastatic pancreatic cancer after a randomised trial proved its clinical and survival benefits over 5-fluorouracil (5-FU) [1]. In subsequent phase III trials of gemcitabine monotherapy, median overall survival (OS) ranged from 5 to 7.2 months, and 1-year survival rates ranged from 11% to 30% [2]. Numerous studies have attempted to increase the efficacy of gemcitabine chemotherapy; however, combining gemcitabine with a variety of cytotoxic and target agents has generally shown no significant survival advantages over gemcitabine monotherapy [2]. To date, randomised trials of two regimens – gemicitabine plus erlotinib [3] and a combination of 5-FU, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) [4] – have demonstrated significant prolongation of OS. However, gemcitabine plus erlotinib resulted in a significant but very small improvement (0.33 months) in median OS (6.24 versus 5.91 months) [3], while the FOLFIRINOX regimen proved quite toxic; 5.4% of patients experienced grade 3 or 4 febrile neutropenia despite 42% of patients receiving support with granulocyte colony-stimulating factor [5]. More effective, better tolerated regimens are therefore required to improve the outcome of patients with advanced pancreatic cancer.

Hyperthermia has been shown to increase the cytotoxic effects of some anticancer agents by facilitating drug penetration into tissues and causing thermal destruction of cancer cells [6, 7]. Gemcitabine has also been shown to be a potent hyperthermic sensitiser in preclinical studies [8]. Moreover, we recently showed that hyperthermia inhibits gemcitabine-induced activation of nuclear factor kappa B (NF-κB), thereby causing enhanced gemcitabine cytotoxicity [9]. These studies suggest that a combination of gemcitabine and hyperthermia may improve the survival of patients with advanced pancreatic cancer. However, few reports exist on combined regional hyperthermia and chemotherapy for advanced pancreatic cancer [10, 11]. This phase II trial aims to evaluate the efficacy and safety of regional hyperthermia combined with gemcitabine in patients with unresectable locally advanced or metastatic pancreatic cancer.

Patients and methods

Patient selection

Patients were enrolled if they fulfilled the following eligibility criteria: histologically or cytologically confirmed adenocarcinoma of the pancreas, existing measurable lesion (more than twice the thickness slice resolution of computed tomography images), over 20 years of age, no other active malignancy, no history of prior chemotherapy or radiotherapy for pancreatic cancer, an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2, sufficient vital organ function (leukocyte count $\geq$4000/mm^3, neutrophil count $\geq$2000/mm^3, platelet count $\geq$100,000/mm^3, haemoglobin $\geq$9.5 g/dL, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leq$90 IU/L, serum total bilirubin $\leq$2 mg/dL, serum creatinine $\leq$1.5 mg/dL, and blood urea nitrogen level $\leq$25 mg/dL), and no serious complications or inadequate physical condition as diagnosed by the physicians.

The exclusion criteria were as follows: interstitial pneumonia or pulmonary fibrosis with radiological findings, active biliary infection, active severe infection, ileus, marked ascites, serious complications, such as severe diabetes mellitus, unstable angina, or myocardial infarction within 3 months of cancer onset, pregnancy or lactation, and a medical history of severe hypersensitivity.

Study design

This multicentre, single-arm phase II study was conducted at six centres. The primary efficacy end point was the 1-year survival rate, and secondary end points were tumour response and safety. A sample size of 18 was required for a one-sided α of 0.1 and a β of 0.2, with an expected 1-year survival rate of 30% and a threshold 1-year survival rate of 10%. This study was approved by the ethics committee of Kyoto Prefectural University of Medicine and was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Clinical Research (the Ministry of Health, Labour and Welfare, Japan). Written informed consent was obtained from all patients. This study is registered in the UMIN Clinical Trials Registry with the identifier UMIN00001221.

Heating methods

Radio frequency (RF) capacitive heating equipment, Thermotron RF-8 (Yamamoto Vinita, Osaka, Japan), operating at a frequency of 8 MHz was employed. This equipment is widely used in Japan and more simple than the radiative devices for regional hyperthermia, using frequencies of about 100 MHz. The thermal profiles in the phantoms demonstrated that one of the advantages of capacitive heating over other heating methods is the depth of heating can be controlled to a certain degree by changing the size of the paired electrodes [12]. One of the well-known disadvantages of a RF-capacitive device is the preferential heating of subcutaneous fat tissue, whereas Asian patients are considered to be relatively suitable because of their slender constitution. The RF energy was transmitted from a generator to two electrodes placed on the

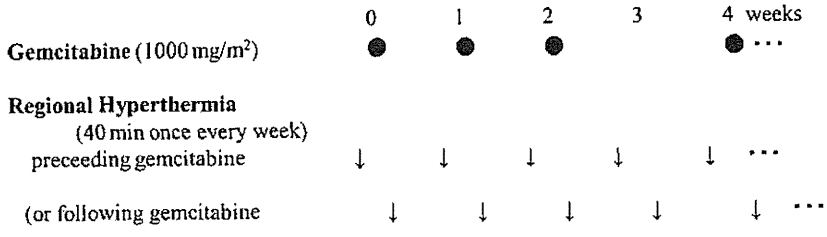

Figure 1. Treatment protocol.

opposite sides of the target area. To improve the coupling of electrodes to the body and avoid skin overheating at the edge of the electrodes, water pads were attached in front of the metal electrodes and temperature-controlled saline solution (2–37°C) was perfused into the water pads. The physical features of Thermotron RF-8 and thermal distribution characteristics in a phantom as well as in the human body when heating with this device have been reported previously [13, 14].

Treatment

One cycle of the chemotherapy regimen comprised a 30-min intravenous infusion of 1000 mg/m^2 gemcitabine once a week for 3 consecutive weeks, followed by 1 week of rest. Patients received hyperthermia therapy for 40 min once every week using a Thermotron RF-8, an 8-MHz capacitive heating device (Yamamoto Vinita).The RF output was increased to the maximum level of the patient's tolerable limit after appropriate adjustments of the treatment setting. The maximum power used by the RF machine ranged from 1100 to 1500 W. Each session was scheduled one day preceding or following gemcitabine administration (Figure 1). We based this schedule on an *in vitro* study in which hyperthermia enhanced gemcitabine cytotoxicity, particularly when it was performed 24 h before or after gemcitabine treatment [9]. This schedule was repeated every 4 weeks until disease progression, unacceptable toxicity, or patient refusal. A second-line chemotherapy regimen for patients who fail first-line therapy was not defined in this study protocol.

In the event of predefined toxic events, protocol-specified treatment modifications were permitted. If patients exhibited a leukocyte count of <3000/mm^3, platelet count of <100,000/mm^3, AST and ALT levels of >100 IU/L, total bilirubin of >5 mg/dL, creatinine level of >1.5 mg/dL, or ≥grade 3 non-haematological toxicity, initiation of the next cycle was postponed until recovery. When patients experienced grade 4 leucopenia for ≥4 days, grade 4 thrombocytopenia, febrile neutropenia with grade 3 neutropenia, or ≥grade 3 non-haematological toxicity, the dose of gemcitabine was reduced to 800 mg/m^2 in the subsequent cycle. Treatment was discontinued if patients required more than two dose reductions, experienced ≥grade 4 non-haematological toxicity or ≥grade 2 interstitial pneumonia, or if the subsequent cycle could not be initiated within 14 days of the last day of gemcitabine administration in the previous cycle.

Assessments

On initiation of every cycle, patient status was assessed according to medical history, physical examination, ECOG performance status, blood counts, and blood chemical tests. CT was performed at baseline and every 4 weeks to evaluate disease progression. Tumour response was determined according to the Response Evaluation Criteria in Solid Tumours (RECIST) [15].

Safety assessments were performed before each cycle according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0), and the worst grade of toxicity was recorded or every patient.

Statistical analysis

Qualitative variables were compared using the chi-square test or Fisher's exact test, and quantitative variables were compared using the student's t-test or a non-parametric Wilcoxon test. All tests were two-sided, and a P value of <0.05 was considered statistically significant. OS was calculated from the date of enrolment until death. Estimation of 1-year survival was performed using the Kaplan–Meier method on an intention-to-treat basis. All analyses were performed using StatView software, version 5.0 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Between November 2008 and May 2010, 18 patients were enrolled; their demographic and baseline disease characteristics are shown in Table 1. Median age was 64 years (range, 47–78 years), and most (72.2%) patients had an ECOG performance status of 0. Carcinoma of the pancreatic head was evident in 61.1%, and 44.4% had biliary stents.