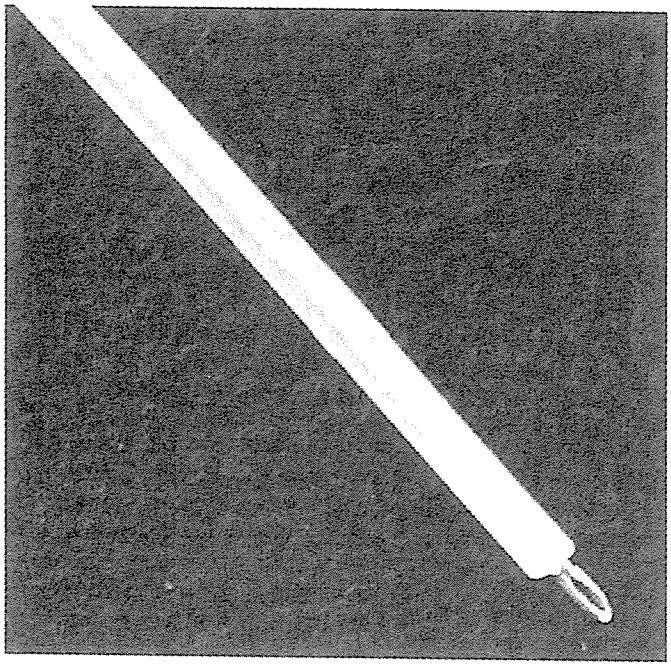

Fig. 1. An electrosurgical snare (thin type, SD-7P-1; Olympus). The length of the snare is adjustable according to the situation. For marking dots, 0.5–1 mm is long enough, but for mucosal incision and submucosal dissection 1–2 mm is the appropriate length.

Procedure of endoscopic submucosal dissection

The therapeutic procedure was carried out as follows (Fig. 2) using a single-channel endoscope (GIF-XQ200, XQ230; Olympus or EG-2931; Pentax, Tokyo, Japan) and a high-frequency generator (Erbotom ICC 200, ERBE, Tübingen, Germany).

1. Marking dots are made using the tip of an electrosurgical snare (thin type) with endocut mode of ICC200 on the circumference of the target lesion (Fig. 2B).
2. Twenty percent glucose (2–3 mL) with a small amount of indigo carmine and epinephrine is injected with a 23-gauge disposal injector needle into the submucosal layer around the lesion to lift it up (Fig. 2C).
3. Incision of the mucosa around the marking dots is made by the tip of an electrosurgical snare (thin type; SD-7P-1; Olympus) with endocut mode to separate the lesion from the surrounding non-neoplastic area (Fig. 2D).
4. Injection of several mL of the above solution is added with the injector needle into the submucosal layer just beneath the lesion (Fig. 2E).
5. The lifting marginated lesion is removed with a standard polypectomy method with another type of snare (normal type, SD-5 L-1; Olympus) if the size of the lesion is appropriate for snaring (Fig. 2F). Or, in the case of larger tumors of more than 2 cm, the submucosa is dissected by the tip of an electrosurgical snare (thin type) with forced mode

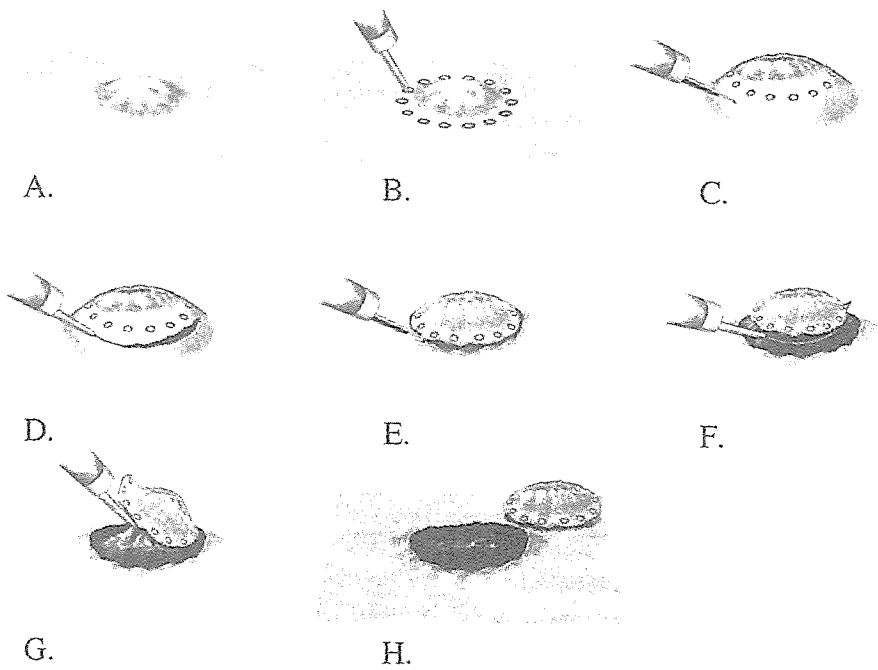

Fig. 2. Schematic drawing of the endoscopic mucosal resection (EMR) procedure using an electrosurgical snare (thin type). A, small elevated lesion without ulceration. B, marking dots are made using the tip of an electrosurgical snare (thin type) on the circumference of the lesion. C, 20% glucose solution (several mL) with a small amount of indigo carmine and epinephrine is injected into the submucosal layer around the lesion. D, Mucosal incision around the marking dots is made by the tip of an electrosurgical snare (thin type) to separate the lesion from the surrounding non-neoplastic area. E, Add several ml of injection solutions into the submucosal layer just beneath the lesion. F, The raised lesion is removed with a standard polypectomy method, if the lesion is appropriate for snaring. G, In the case of a larger tumor, dissect the submucosal layer using the tip of a snare (thin type) as a flexible diathermic knife until removal. H, Artificial ulcer is carefully examined for a residual tissue, visible vessels, and a perforating hole. Retrieve the resected specimen with grasping forceps.

at 40–60 W until the lesion is completely cut off from the gastric wall without snaring (Fig. 2G).

6. Finally, the resected specimen is retrieved with grasping forceps and subjected to histopathological examination (Fig. 2H).

Histological evaluation

The resected specimens were fixed with formalin and cut into 2 mm slices, then embedded in paraffin. A histological section was made from each block and stained with hematoxylin eosin. Histological assessment was microscopically performed in detail according to the Japanese Classification of Gastric Carcinoma.[8] As submucosal invasion, existence of undifferentiated-type cells, and/or vessel infiltrations are regarded as high risks for lymph node metastasis, surgical intervention was strongly recommended.[9] Evaluation of the extension of cancer cells to the lateral margin was classified into the following three groups.

1. Complete resection: free of cancer glands on cut ends.
2. Incomplete resection: exposition of cancer glands on cut ends.
3. Not evaluable: impossibility of evaluation due to burn effect by diathermic treatment, mechanical damage or piecemeal resection.

Assessment of therapeutic efficacy

En-bloc resection

En-bloc resection was defined when the resected tumor was confined to a single resected specimen with complete resection as defined above. Even in those treated with resection of two or more steps, it was defined as an en-bloc resection when one of the resected specimens contained the whole tumor in a single piece and the other resected specimens did not contain any cancer glands histologically.

Complications: Bleeding and perforation

Bleeding was defined as massive bleeding during the procedure that required blood transfusion, or postoperative bleeding that required hemostatic treatment, such as endoscopic clipping, thermocoagulation and/or injection therapy. Perforation was diagnosed endoscopically when the other abdominal organs, mesentric fat or intra-abdominal space were observed during the procedure and/or by the presence of free air on a plain abdominal X-ray.

Table 1. Clinicopathological features of the subjects

Mean size (range)		29 mm (5–85)
Location	C/M/A	11/20/28
	LC/GC/AW/PW	22/8/12/17
Macroscopic type	I	3
	IIa	15
	IIa + IIc	5
	IIc	36
Histology	Differentiated	58
	Undifferentiated	1
Depth	Mucosa	55
	Submucosa	4
Vessel infiltration	Presence	2
	Absence	57

A, antrum; AW, anterior wall; C, cardia; GC, greater curvature; LC, lesser curvature; M, gastric body; PW, posterior wall.

RESULTS

Clinicopathological features

Endoscopic resection was completed in the entire lesions and histological examination was performed in every resected specimen. Table 1 summarizes the clinicopathological features of the lesions treated with the new technique. The sizes of the lesions were 5–85 mm (mean size: 29 mm) in the greatest diameter. The maximum size of the resected specimen was 90 × 75 mm and complete en-bloc resection was achieved without any complications despite its huge size (Fig. 3). Tumor location varied from cardia to antrum and the bias of the distribution was not observed. Among the successfully resected lesions, there was a large tumor more than 4 cm located in cardia to esophagogastric junction, which seemed impossible to resect with a conventional EMR method (Fig. 4).

Through histological evaluation, one lesion was diagnosed as having a component of undifferentiated-type adenocarcinoma and four lesions revealed submucosal invasion. Among them, vessel infiltration was observed in two lesions. Those two patients with submucosal invasion plus vessel infiltration had additional gastrectomy with lymph node dissection, which revealed no residual cancer or lymph node metastasis. The other lesions with minute invasion into the submucosa were closely followed without additional surgery, because a recent study revealed that the risk for lymph node metastasis of such lesions was quite low.[9]

En-bloc resection rate

Among 59 lesions, 56 lesions (95%) were resected completely in an en-bloc fashion regardless of their size and location. Only three lesions (5%) were not resected as en-bloc resection. One lesion, which was located in the cardia, was resected in three pieces, although completely resected. The others were resected in a single piece, but the histological evaluation revealed that the cancer margin was not evaluable due to the burn effect by diathermic treatment in one lesion and, in the other lesion, resection was incomplete due to mismarking of the cancer margin. Follow-up endoscopy with biopsy revealed no residual cancer cells in these patients and they were followed without additional treatment.

Complications

Minor bleeding was encountered in all the lesions when incising the mucosa or dissecting the submucosa, but complete hemostasis was achieved within a few minutes with thermocoagulation using hemostatic forceps. Massive bleeding requiring blood transfusion was not observed. Bleeding a day after the procedure was experienced in one case (1.7%), which was noticed by hematemesis. Emergent endoscopy revealed bleeding from the vissible vessel on the ulcer bed and hemostasis was achieved with hemoclips. Perforation was observed in two cases (3.4%). In one case, no perforated

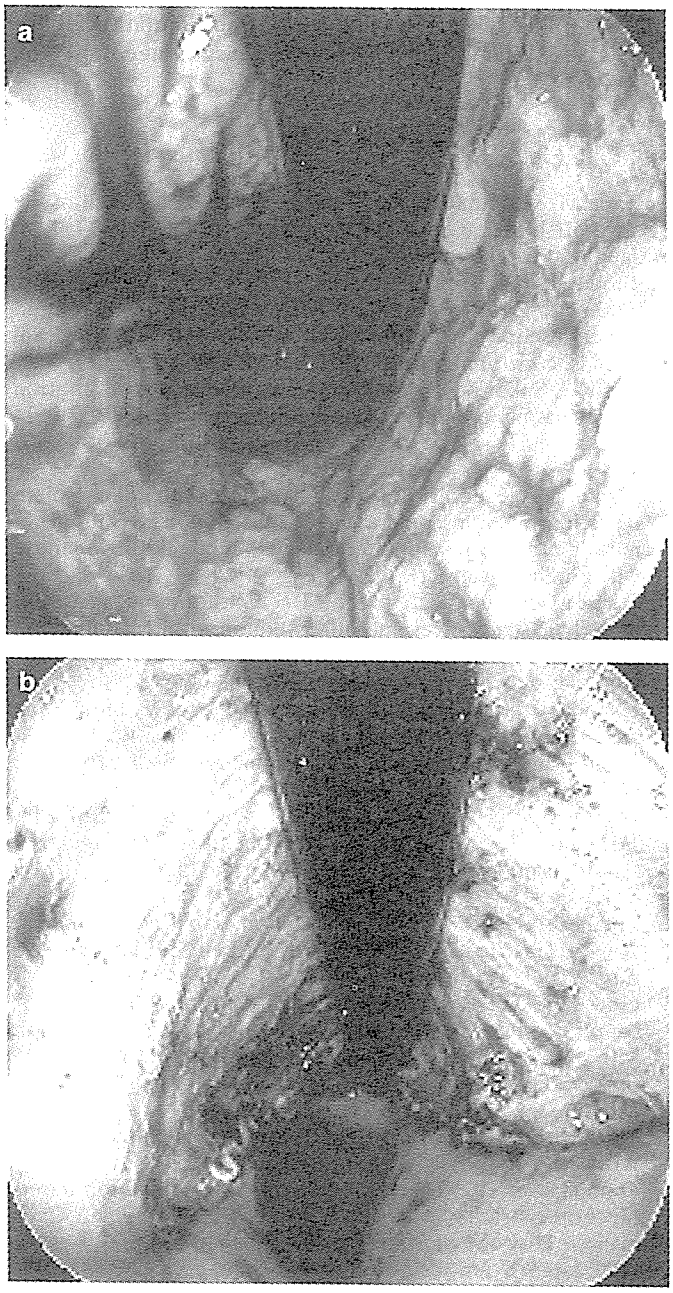

Fig. 3. A large superficial adenocarcinoma, spreading over the entire lesser curvature of gastric body from cardia to gastric angulus. The lesion was resected completely en bloc, in spite of its huge size.

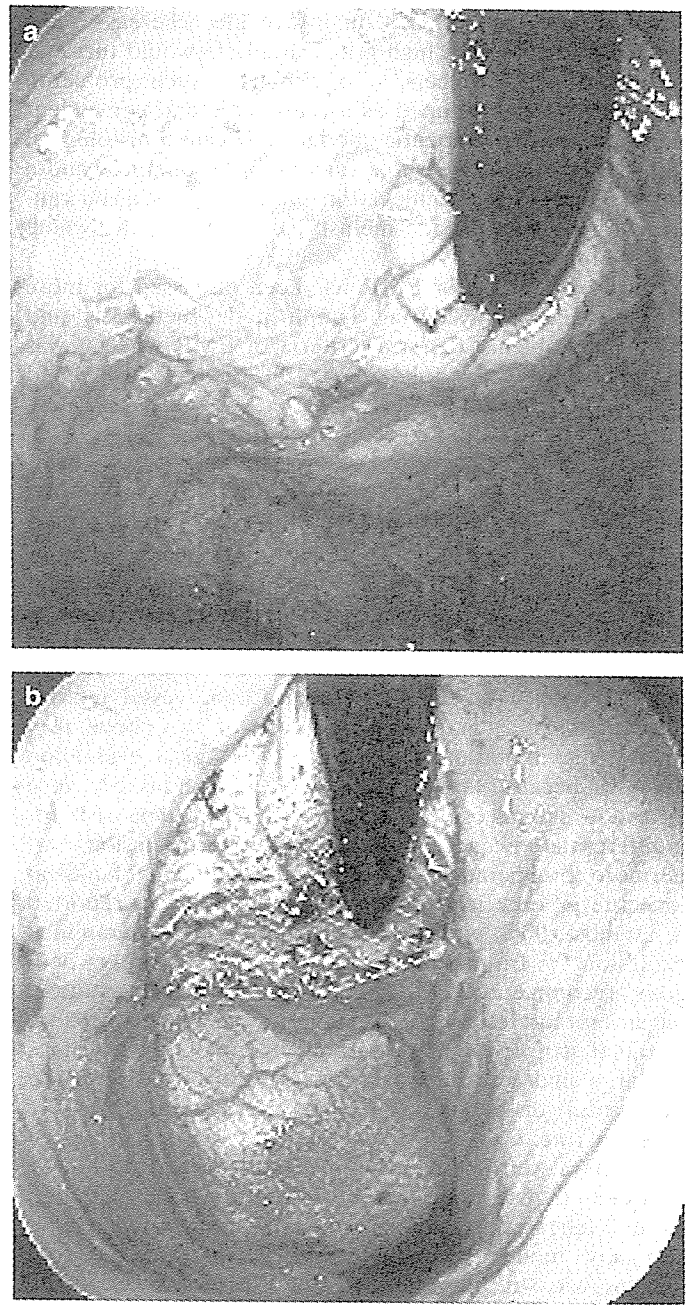

Fig. 4. A large IIa lesion located in a difficult area for endoscopic treatment. The lesion is distributed from the esophagogastric junction to cardia. The lesion was resected completely en bloc, despite its difficult location.

hole was noticed during the procedure, but the abdominal X-ray on the next day revealed free air, probably due to microperforation. The patient had no symptoms and recovered well with 3 days of fasting and antibiotics administration. In the other case, a small perforated hole was closed with hemoclips immediately, and the patient discharged uneventfully at 7 days after the treatment.

DISCUSSION

Endoscopic mucosal resection has been developed mainly in Japan and not in Western countries, probably because the incidence of gastric cancer and the tumor description[10] are different between them. Although decreasing in number, the incidence of gastric cancer is approximately 80 patients per 100 000 population in Japan and nearly half of the patients have early gastric cancer,[11] which have high probabilities of no lymph node or no distant metastases. Previously, before EMR was introduced, all patients had to be treated with open surgery in Japan, even if the tumor was an intramucosal cancer without lymph node metastasis. In contrast, in Western countries, those tumors have been diagnosed probably as a high-grade dysplasia and followed without treatment.[10] Both situations might have led to unfortunate results, as unnecessary gastrectomy in Japan

would reduce the patient's quality of life, whereas in the West, the tumors with high-grade dysplasia would turn out to be advanced cancers.[12] To eliminate such problems, EMR has been developed as a reasonable and convenient diagnostic and therapeutic modality, because histological information about the whole tumor can be obtained and a curative treatment is achieved in the case of localized cancers without lymph node metastasis, preserving the whole gastric function.

Patient eligibility for EMR has been discussed for more than 10 years, considering lymph node metastasis and technical problems. Classical criteria of EMR when it was introduced were as follows: (i) differentiated, elevated type less than 2.0 cm in diameter; (ii) differentiated, depressed type without ulceration, less than 1.0 cm in diameter; and (iii) undifferentiated, depressed type without ulcer formation, 0.5 cm in diameter.[13] However, from the point of lymph node metastasis, a recent study of surgically resected cases at two reliable large special cancer centers in Japan, reported that the expansion of EMR criteria was, at least, possible as the following: (i) intramucosal cancer of differentiated type, without ulcer findings or vessel infiltration; and (ii) intramucosal cancer of differentiated type, with ulcer findings, without vessel infiltration, less than 3 cm in size.[9] In case of the above new criteria, the importance of precise histological evaluation increases, because larger tumors have an increased chance to invade submucosa or vessels and have some risk for undifferentiated cancer involvement within the lesion. Furthermore, preoperative prediction of fulfillment of indication criteria, especially in tumor depth, has been reported as, at most, 90% whenever an expert at EUS examined the lesion.[14,15] En-bloc resection defined as above is definitely recommended for precise histological evaluation, but an en-bloc resection may never be achieved with conventional strip biopsy or aspiration methods if the lesions are large and/or located in difficult areas. Although the submucosal dissection technique increased the en-bloc resection rate, even in difficult cases, the disadvantages of this method were reported as the high complication rates. In order to overcome these disadvantages, especially to prevent perforation, the IT-knife was introduced, but it had only modest benefits on perforation, and severe bleeding was still observed in 22% of cases.[7] In our study, endoscopic submucosal dissection using the tip of an electrosurgical snare (thin type) enabled us to resect larger tumors, with fewer complications, maintaining a high en-bloc resection rate, even if they were nearly 10 cm in the greatest diameter, and the tumors were located in difficult areas for endoscopic treatment, such as the esophagogastric junction and the cardia. The merit of the thin-type snare is that the length of the tip is adjustable to control the depth of incision, which prevents perforation. Furthermore, its easy maneuverability due to the soft and flexible nature enables us to cut in any direction. We believe that this technique is very promising and will break through technical problems that have caused problems for a long time, although further accumulation of the treated cases in our and other institutions are needed.

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Comparison of Various Submucosal Injection Solutions for Maintaining Mucosal Elevation During Endoscopic Mucosal Resection

Background and Study Aims: One of the major complications of endoscopic mucosal resection (EMR) for gastrointestinal tumors is perforation, and the most effective way of preventing perforation is to elevate the lesion sufficiently by endoscopic injection of fluid into the submucosa.

Materials and Methods: In order to compare the lesion-lifting properties of several different solutions, 1 ml of each of the following solutions was injected into the submucosa of the resected porcine stomach: normal saline, 3.75% NaCl, 20% dextrose water, 10% glycerin with 0.9% NaCl plus 5% fructose, and two sodium hyaluronate (SH) solutions.

Results: Significantly higher initial elevation was produced by both SH solutions, and it remained higher than that achieved by the other solutions at all times. Hypertonic solutions, especially 10% glycerin with 0.9% NaCl plus 5% fructose, tended to produce and maintain greater mucosal elevation than normal saline, but the difference was not significant.

Conclusions: SH solutions were the most suitable ones for producing and maintaining long-term mucosal elevation, while the superiority of hypertonic solutions over normal saline was not clearly demonstrated.

Introduction

The technique of endoscopic mucosal resection (EMR) was developed to provide less invasive treatment for gastrointestinal tumors [1–4]. Since the introduction of the submucosal dissection technique in Japan, the indications for EMR have recently been extended to larger tumors. However, the problem of a high complication rate has emerged. When an IT knife is used to treat gastric tumors, a 5% perforation rate has been reported [5]. If perforation occurs, the EMR procedure has to be interrupted immediately in order to close the perforation and prevent severe peritonitis. The most effective and simple way of preventing perforation is to maintain a sufficiently thick submucosal layer by endoscopic injection of fluid into the submucosa. Although various solutions for submucosal injection, such as hypertonic saline [6], dextrose water [7], glycerin solution [8], and sodium hyaluronate [9], have been used, as well as normal saline [10], differences between these solutions with regard to their ability to produce and maintain mucosal elevation have not been assessed, and the choice mainly depends on the operators' preferences. The present study compared changes in the mucosal elevation over time after submucosal injection and assessed which of the available solutions is the most suitable for producing and maintaining mucosal elevation.

Materials and Methods

Porcine stomachs were used for this study within 2 h after resection. The thickness of the gastric wall varies among different parts of the organ. The upper third of the stomach, which is similar to the human stomach, was therefore used (Figure 1). The

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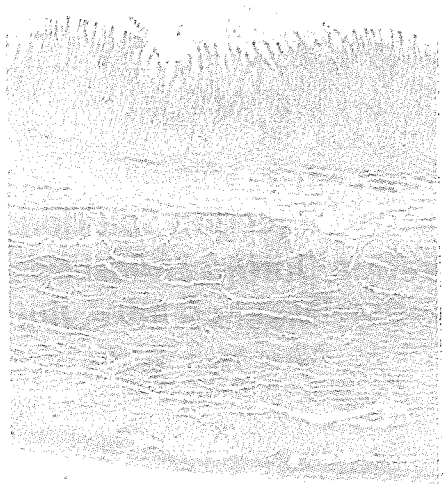

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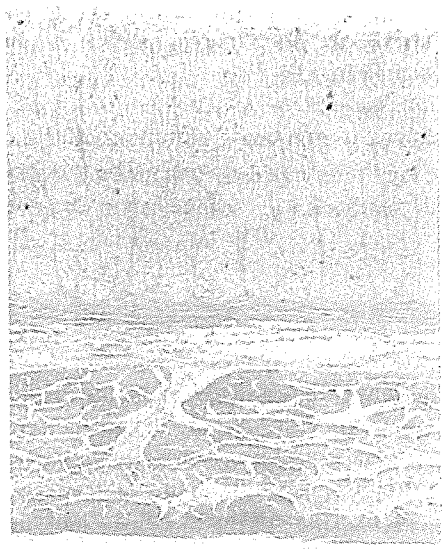

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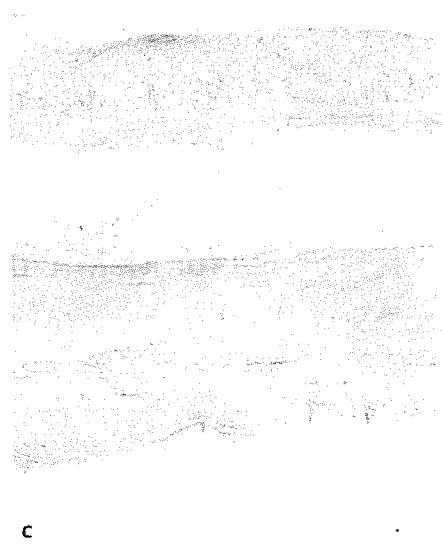

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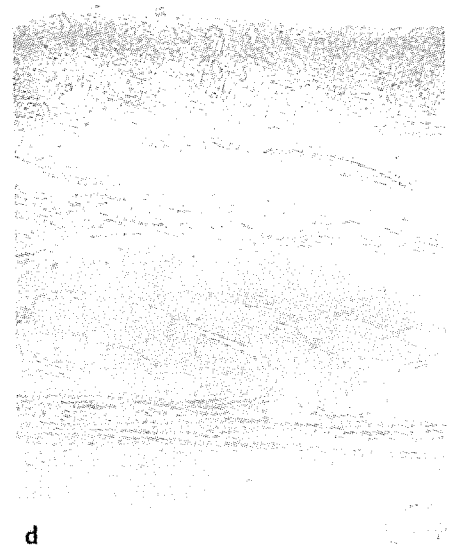

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Figure 1 The porcine and human stomachs (hematoxylin-eosin stains, original magnification × 20). The wall thickness varies among different parts of the organ in the porcine stomach; the upper third of the porcine stomach is similar to the human stomach.
a The lower third of the porcine stomach.
b The middle third of the porcine stomach.
c The upper third of the porcine stomach.
d The human stomach.

gastric specimen was cut into approximately 5 × 5 cm squares and stretched flat on a cork board with pins. Using a small syringe (2.5ml) and a 23-gauge needle, 1 ml of each solution was horizontally injected into the submucosa from the margins of the specimen (Figure **2a**). The volume and course of the injections were determined by a pilot study to assess the appropriate settings with slight variation. Although 5–20 ml or more of injection solutions are currently used in clinical practice, it was considered that the small volume was appropriate and sufficient to assess the ability to produce and maintain mucosal elevation. Horizontal injections were carried out not from the mucosal surface but from the cut surface, as this made it possible to achieve constant and reproducible injections and minimized the volume loss from the stitching site. The solutions examined were:

- 0.9% NaCl (normal saline, NS) [10].
- 3.75% NaCl (hypertonic saline, HS) [6].
- 20% dextrose water (DW) [7].
- 10% glycerin with 0.9% NaCl plus 5% fructose (Glyceol; Chugai Pharmaceutical Co., Tokyo, Japan) [8].
- Two solutions of sodium hyaluronate (SH) with different mean molecular weights (an 800 kDa preparation: Artz, Kaken Pharmaceutical Co., Tokyo, Japan; and a 1900 kDa preparation: Suvenyl, Chugai Pharmaceutical Co., Tokyo, Japan) [11,12], which are already in use for submucosal injection in clinical practice.

With respect to the SH solutions, the tested solutions were diluted with normal saline to 0.5% and 0.25% concentrations of SH in an 800kDa solution and a 1900 kDa solution, respectively. These concentrations were earlier found to be appropriate for endoscopic injection in another study in which we measured the actual endoscopic injection pressure generated by a 21-gauge endoscopic injection needle, as well as the viscoelasticity [13].

After each solution had been injected into the submucosal layer, mucosal elevation was observed from the lateral direction and recorded with a measuring device immediately and 5, 10, 15, 20, 25, 30, 45, and 60 min after injection (Figure **2b**). In order to en-

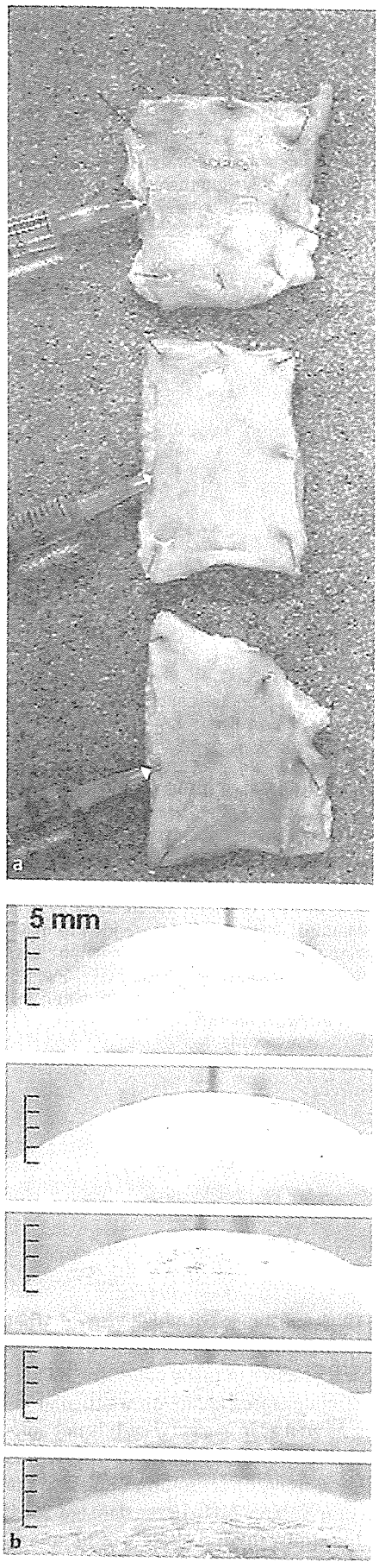

Figure 2 Submucosal injection of various solutions. **a** Submucosal injections of 1 ml of each solution were made horizontally from the margins of gastric specimens stretched flat on a cork board with pins. **b** Chronological changes in the mucosal elevation are shown from top to bottom in turn (immediately after injection and 5, 15, 30, and 60 min after injection).

sure that the position of the specimen and the camera relative to each other remained constant and that the pictures were taken from absolutely the same angle, the specimens were set at the same distance between the edge of the fixed cork board and the fixed camera (CAMEDIA C-200 Ultra Zoom, Olympus, Tokyo, Japan) on a tripod. The recorded pictures were analyzed using a personal computer, and the mucosal elevation at each time was measured by image analysis software (WinROOF version 3.51, Mitani Co., Fukui, Japan). The same experiment was repeated five times for each group, and the mean mucosal elevation at each time was compared among the solutions. To ensure that the experiment was conducted in a blinded fashion, the solutions examined were numbered from 1 to 6, and the names of solutions were only disclosed after measurements of the mucosal elevation. This experiment was carried out by three investigators, who individually performed the injections, took the photographs, and measured the mucosal elevation using a personal computer. Statistical analysis was carried out with Student's or Welch's *t*-test using SAS software (SAS Institute, Inc., Cary, North Carolina, USA). A *P* value of less than 0.05 was considered statistically significant.

Results

The initial mucosal elevation produced by submucosal injection and the chronological changes are shown in Figure 3 and 4, respectively. Similar initial elevation was produced by the two SH solutions, and the elevation created by each SH solution was significantly greater ($P < 0.05$) than that produced by NS, HS, and 20% DW. There were no significant differences among the other solutions, although NS tended to produce less elevation than three hypertonic solutions and Glyceol tended to produce greater elevation than the other hypertonic solutions.

When assessed over time, similar changes in elevation were observed between the two SH solutions, and both SH solutions maintained a greater degree of elevation than the others at all times. The elevation produced by NS was less than that of the three hypertonic solutions at all times, but there was no significant difference.

Discussion

Until recently, it was only possible to carry out EMR in smaller tumors, using the cap technique or the grasping method [14–17], since the size of the specimens that could be removed during a single EMR procedure was limited (approximately 10–20 mm, depending on the tumor location and the operators' skill). However, most large mucosal tumors in the gastrointestinal tract are localized lesions without lymph-node metastases [1,3,18]. The submucosal dissection technique introduced by Hirao et al. [6] has subsequently been improved by several investigators [7,19,20], allowing en-bloc resection of larger tumors. En-bloc resection of the whole tumor with free margins should be carried out when possible, as it is essential for precise evaluation of the tumor histology and completeness of the resection in order to prevent residual tumor or recurrence. However, the high complication rate associated with this method has prevented more

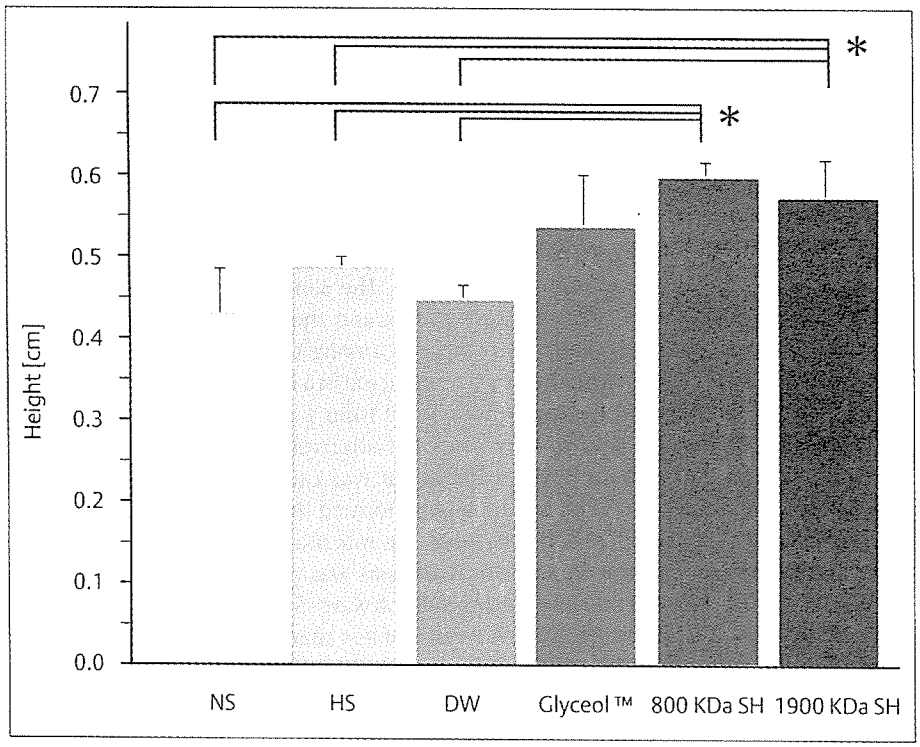

Figure 3 The initial mucosal elevation after submucosal injection of various solutions. Both sodium hyaluronate solutions produce significantly greater elevation than normal saline (NS), hypertonic saline (HS), or 20% dextrose water (DW) ($^*P < 0.05$). Glyceol produced greater elevation than the other hypertonic solutions and normal saline produced less elevation than the hypertonic solutions, but there were no significant differences.

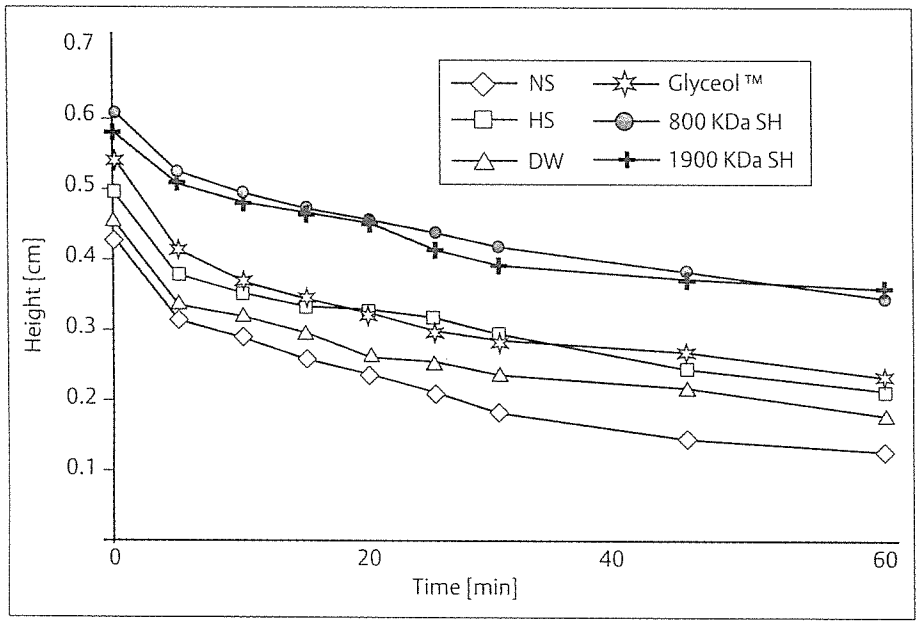

Figure 4 Chronological changes in the mucosal elevation after submucosal injection of various solutions. Both sodium hyaluronate solutions maintained a greater degree of mucosal elevation at all times than the other solutions. Hypertonic solutions maintained an intermediate degree of mucosal elevation between those achieved by normal saline and sodium hyaluronate. No differences were evident between the three hypertonic solutions, although Glyceol tended to maintain a greater mucosal elevation until 10 min after injection. NS: normal saline; HS: hypertonic saline; DW: dextrose water; SH: sodium hyaluronate.

widespread use of EMR in the treatment of larger tumors [5]. The lesion-lifting properties of the different solutions used for submucosal injection are therefore an important issue.

This study showed the superiority of the sodium hyaluronate (SH) solutions tested over the other submucosal injection solutions for producing and maintaining mucosal elevation. The two types of SH solution tested, with similar injection pressure and viscoelasticity, showed a similar ability to produce and maintain mucosal elevation – showing that the lesion-lifting properties of SH solutions are affected by the concentration and molecular weight of SH.

Sodium hyaluronate is a thick substance with high viscosity that is widely found in connective tissues. The current approved indications for its use in clinical practice in many countries, including Japan, Europe and the United States, are for intra-articular injections for osteoarthritis, as well as in eye surgery. It is not antigenic or toxic in humans [21 – 23], and only minor adverse effects have been reported in clinical use [24]. SH solutions are also the best for submucosal injection with regard to tissue damage, since they are isotonic with extracellular fluid; the safety of submucosal injection was confirmed in a previous study [20]. The single (but crucial) disadvantage of SH solutions may be their high cost, which may mean that not all tumors need to be treated

with SH solutions. The use of hypertonic solutions should not be abandoned in clinical practice; this study shows that they tend to produce and maintain greater mucosal elevation than normal saline.

Among the hypertonic solutions, Glyceol may be preferable, as it produces greater mucosal elevation than the other hypertonic solutions. However, when hypertonic solutions are used, attention needs to be given to the potential tissue damage. Extensive ulceration has often been observed after injection therapy for endoscopic hemostasis when 10% saline or 50% dextrose water is used. Increased tissue damage may also make it difficult to obtain a precise histological diagnosis of the resected specimens and may cause delayed ulcer healing after EMR.

Although the present study demonstrated clear differences among the various submucosal injection solutions used for EMR, it has some limitations. The major limitation is that the influence of blood flow, body temperature, peristalsis, and absorption from the tissue in a living stomach were not assessed, as the study was conducted in resected specimens. A similar experiment was previously performed using a live porcine stomach, but achieving reproducible mucosal elevation in the same conditions and precise measurement of the height of elevation were very difficult in the in-vivo setting. Resected stomach was therefore used in this study, since the reproducibility of the mucosal elevation and its precise measurement were much more important than the above factors that affect a live stomach. In addition, it was considered that these factors would be likely to influence all of the solutions in the same way. The model used in this study is therefore an appropriate one for an investigation comparing the ability of different submucosal injection solutions to produce and maintain mucosal elevation, although the mean time for the mucosal elevation to flatten out may be somewhat different from that in a living stomach.

Conclusion

The most suitable solutions for submucosal injection were sodium hyaluronate solutions, which produced and maintained greater mucosal elevation for a long period. These solutions should be used as the first-line solutions for submucosal injection during EMR, particularly in difficult situations and those involving larger tumors.

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Different Mixtures of Sodium Hyaluronate and Their Ability to Create Submucosal Fluid Cushions for Endoscopic Mucosal Resection

Background and Study Aims: Sodium hyaluronate (SH) is a promising submucosal injection solution during endoscopic mucosal resection, but its high cost is an obstacle to more widespread use. The aim of this study was to identify an appropriate low-cost SH solution by varying the molecular weight of SH and mixing various solutions with it.

Materials and Methods: The viscoelasticity of various SH solutions was first measured. The concentrations of two 1% SH preparations with different molecular weights (800 kDa and 1900 kDa) were adjusted to 0.5%, 0.25%, and 0.125%, using 0.9%/3.75% normal saline (NS), 5%/20% dextrose water (DW), and a glycerin solution (Glyceol): 10% glycerin with 0.9% normal saline plus 5% fructose. The ability of these SH solutions to create submucosal fluid cushions (SFCs) was then investigated in the stomachs of two live minipigs.

Results: The 0.25% 1900 kDa SH/NS solution and the 0.125% 1900 kDa SH/20% DW solution created a similar viscoelasticity to that of the 0.5% 800 kDa SH/NS solution. The ability of these solutions to create SFCs was also similar. In addition, the 0.125% 1900 kDa SH/Glyceol solution created similar SFCs, with a synergistic effect of increased viscoelasticity and the hypertonic nature of glycerin.

Conclusions: A mixture of higher molecular weight sodium hyaluronate with a sugar solution (particularly 20% dextrose), with or without glycerin, should be regarded as a cost-effective option for creating SFCs instead of the conventional SH solution made with the same amount of a 1% 800 kDa SH preparation and normal saline.

Introduction

One of the major complications of endoscopic mucosal resection (EMR) is perforation [1,2]. The most effective way of preventing perforation is to create an adequate submucosal fluid cushion (SFC) between the lesion and the muscle layer by submucosal injection. Among various solutions proposed for submucosal injection during EMR [3–10], it is reported that the most suitable is sodium hyaluronate (SH) [11,12]. Sodium hyaluronate is a thick substance with high viscoelasticity that is widely found in connective tissues. The current approved indications for its use in clinical practice in many countries, including Japan, Europe and the United States, are for intra-articular injections for osteoarthritis, as well as in eye surgery. It is not antigenic or toxic in humans [13–15], and only minor adverse effects have been reported in clinical use [16]. However, there are the three major disadvantages of SH for use as a submucosal injection solution during EMR: its high cost, specific storage requirements, and the need to reconstitute it for use in a solution. Its most important and crucial disadvantage is its high cost; the best way of achieving the maximum lesion-lifting effect at the lowest cost therefore needs to be elucidated. This study investigated the comparative performance of different SH mixtures to identify the appropriate submucosal injection solutions of SH in terms of viscoelasticity and the ability to create SFCs.

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Materials and Methods

Measurement of Viscoelasticity

Two 1% SH preparations with different molecular weights available in Japan were used – an 800-kDa preparation (Artz, Kaken Pharmaceutical Co., Tokyo, Japan) and a 1900-kDa preparation (Suvenyl, Chugai Pharmaceutical Co., Tokyo, Japan). These SH preparations were diluted with various other solutions, and 10-ml volumes of different mixtures were prepared. Analysis of the viscoelasticity of each SH solution was carried out on a controlled stress rheometer (RheoStress 300, Thermo Haake Ltd., Germany), using a parallel plate with a diameter of 60 mm. The instrument was operated in the dynamic mode at 5% strain with a frequency range of 0.01 – 100 Hz at 37 C. Five milliliters of each solution was used per measurement, and two measurements were made for each solution. The parameters measured were the storage and loss shear modulus, G′ and G″, and the mean complex shear modulus was calculated for comparison between the various SH solutions (see formular below).

$$G^* = \sqrt{(G'^2 + G''^2)}$$

In the first investigation, the viscoelasticity of two 1% SH preparations mixed with various volumes of 0.9% NaCl (normal saline, NS) was compared in order to assess the influence of different molecular weights. The concentrations were adjusted to 1%, 0.5%, and 0.25%.

In the next investigation, the viscoelasticity of various SH solutions mixed with 5% dextrose water (DW), 3.75% NaCl (hypertonic saline, HS), 20% DW, or 10% glycerin with NS plus 5% fructose (Glyceol, Chugai Pharmaceutical Co., Tokyo, Japan) was compared in order to assess the best solution for mixture with SH. These solutions are commonly used for submucosal injections [5 – 7], with the exception of 5% DW, which was chosen as a control solution when sugar solutions were compared with saline solutions. The concentrations were adjusted to 0.5%, 0.25%, and 0.125%.

Ability to Create SFCs in Living Stomachs

After the viscoelasticity measurements, the feasibility of various SH solutions for use as submucosal injection solutions was investigated. Endoscopy was carried out with standard endoscopes (Olympus GIF-XQ230; Olympus Corporation, Tokyo, Japan, and Pentax EG-2931, Pentax Corporation, Tokyo, Japan) in two overnight fasted minipigs (*Sus scrofa*; Miniature Swine, CSK Research Park, Inc., Nagano, Japan) placed in the left lateral decubitus position after tracheal intubation and induction of general anesthesia. A disposable 23-gauge catheter injection needle (Olympus NM-200L-0423) was used to inject 2 ml of each solution into the submucosal layer at separate sites in the stomachs. If the mucosa did not elevate after 0.5 ml of injection, the needle was repeatedly reinserted at different sites until two successful SFCs per group were created. Solutions with a viscoelasticity similar to that of a 0.5% 800 kDa SH solution made with NS were selected as the test groups from the viscoelasticity results, as a high success rate with curative EMR was reported in a study using this SH solution [17]. NS was also tested as a control solution. All of the solutions were mixed with a minimal volume of indigo carmine dye (approximately 0.5 ml per 10 ml of solution) so that the submucosal diffusion could be visualized. The endoscopes were kept in the stomach to allow observation of the SFCs for up to 30 min. When endoscopic observations were completed, Endoclips were placed at a distance of 1 cm from the injection sites, and the minipigs were allowed to recover from the anesthesia. Each procedure was recorded on videotape, and endoscopic photographs were taken. After 1 week, the minipigs were sacrificed and the stomachs were retrieved for histological evaluation. The stomachs were stretched flat on a cork board with pins and fixed with formalin, cut at the separate injection sites and embedded in paraffin. Histological sections were made from each block and stained with hematoxylin and eosin, and the effect of the injections on the tissue was examined microscopically.

Results

Measurement of Viscoelasticity

When two SH preparations with different molecular weights were compared, the viscoelasticity of 1% and 0.5% 800 kDa SH solutions corresponded to that of the 0.5% and 0.25% 1900 kDa SH solutions, respectively, when they were diluted with normal saline (Figure **1**). These findings showed that the viscoelasticity of the 800 kDa SH solutions was nearly the same as that of 1900 kDa SH solutions that contained half the concentration.

When various SH solutions mixed with different solutions were compared, sugar solutions (5% and 20% DW and Glyceol, which contains 5% fructose) were found to have greater viscoelasticity than the saline solutions (NS and HS) (Figure **2**). Although the differences between the sugar solutions were not large, the addition of 20% DW produced greater viscoelasticity than the other sugar solutions. As a result of increasing viscoelasticity, a

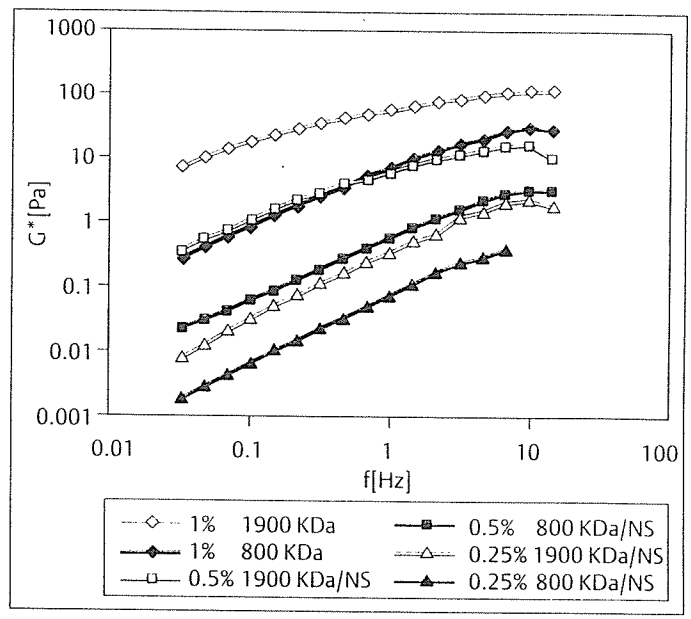


Figure **1** The viscoelasticity of sodium hyaluronate solutions mixed with normal saline. Comparison between two sodium hyaluronate (SH) preparations (800 kDa and 1900 kDa) showed that the viscoelasticity of the 800 kDa SH solutions was similar to that of 1900 kDa SH solutions at half the concentration. G*: mean complex shear modulus; f: frequency; NS: normal saline.

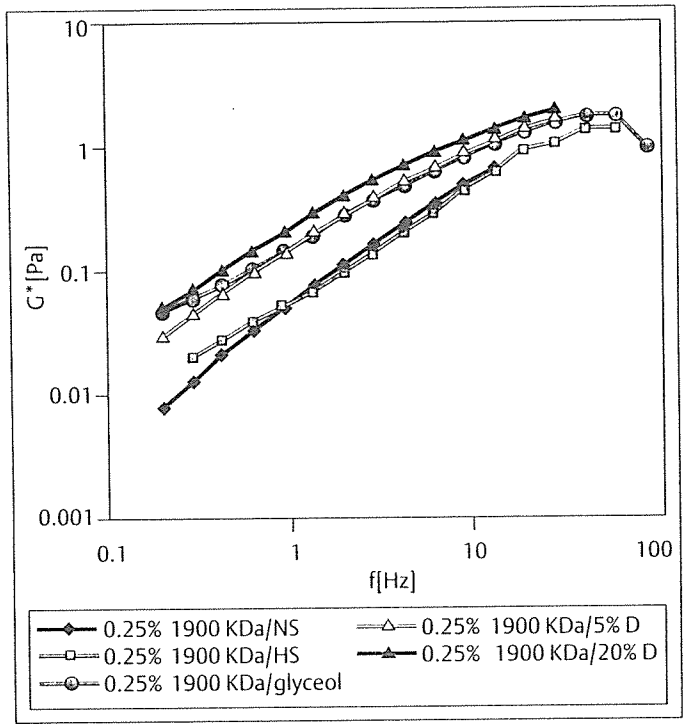


Figure 2 The viscoelasticity of 0.25% 1900 kDa sodium hyaluronate (SH) solutions. The compared solutions were made by mixing a 1% 1900 kDa sodium hyaluronate preparation and three times the volume of normal saline, hypertonic saline, Glyceol, 5% dextrose water, or 20% dextrose water. The saline solutions and sugar-containing SH solutions produced greater viscoelasticity than saline-containing SH solutions. Among the sugar solutions, 20% dextrose water produced greater viscoelasticity than the other sugar solutions. G*: mean complex shear modulus; f: frequency; NS: normal saline; HS: hypertonic saline; D: dextrose water.

0.125% 1900 kDa SH solution made with 20% DW produced a similar viscoelasticity to that of a 0.25% 1900 kDa SH solution made with NS.

Ability to Create SFCs in Living Stomachs

On the basis of the above viscoelasticity measurements, the following three SH solutions were selected for testing of their ability to create SFCs:

- A 0.5% 800 kDa SH solution made with normal saline (NS).
- A 0.25% 900 kDa SH solution made with NS.
- A 0.125% 1900 kDa SH solution made with 20% dextrose water.
- In addition, a 0.125% 1900 kDa SH solution made with Glyceol was also tested, as the synergistic effect of the increased viscoelasticity of SH and the hypertonic potency of glycerin might be expected to result in better SFCs.

For all of the above solutions, successful complete fluid injections, with no failed injections, were made to create SFCs, with two separate punctures per group. Endoscopic observation revealed that similar SFCs were initially created by all of the tested solutions except for NS and that they persisted for up to 30 min, although the height of the mucosal elevation declined over time (Figure 3). By contrast, the height of the mucosal elevations created by NS was apparently less than that created by the other solutions, and the corresponding SFCs flattened out within 10 min. Histological evaluation of the resected stomachs showed no tissue damage in any of the injected sites.

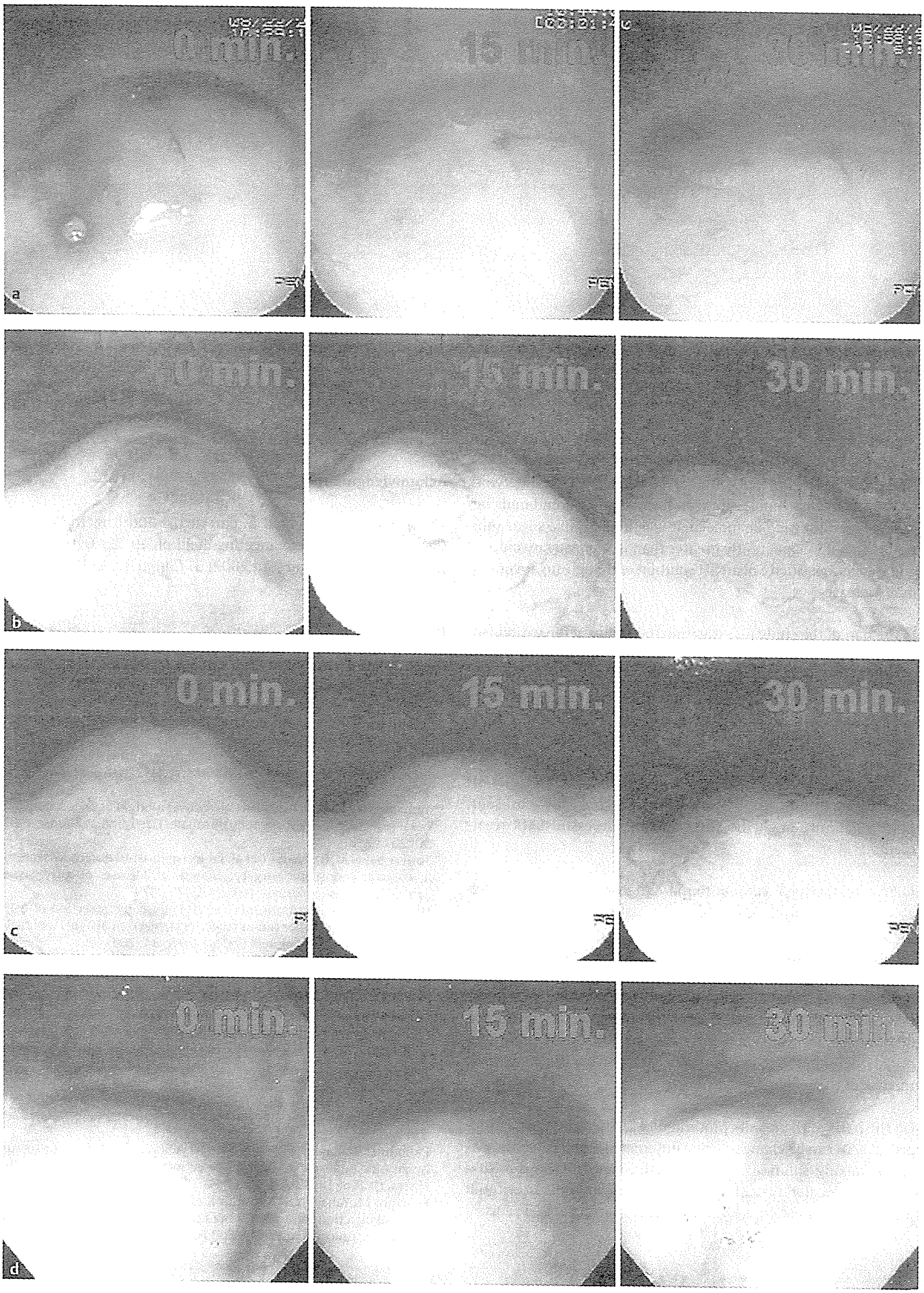
Discussion

Endoscopic mucosal resection developed as a less invasive method of treating gastrointestinal tumors, and led to a considerable improvement in the patients' quality of life [18]. Several research groups introduced more advanced EMR methods using cutting devices, providing endoscopic submucosal dissection techniques [1,2,6–10,17], and larger tumors can now be resected endoscopically with adequate margins. Although these techniques initially attracted considerable interest, high complication rates due to bleeding and perforation became a problem [1,2]. In order to prevent perforation, an SFC is created to elevate the lesion from the muscle layer. Previous studies have shown that, among the submucosal injection solutions available, those containing sodium hyaluronate are by far the best [11,12]. A 0.5% 800 kDa SH solution made with NS has generally been used by Japanese endoscopists, as its viscoelasticity enables it to pass through a 23-gauge injection needle and create an adequate SFC [8,9,17].

Although SH should be used as the first-line injection solution for submucosal injection in clinical practice, a factor that needs to be considered is that SH is also far more expensive than other solutions. In Japan, the prices of both 1% SH preparations are around US $ 10/ml; the total cost of creating an SFC for each tumor resection would therefore be around $ 100 or more with a 0.5% 800 kDa SH solution, since a fluid injection of 20 ml or more is needed to resect each tumor. By contrast, other solutions such as saline/dextrose solutions or Glyceol are available for $ 0.01–$ 0.03 /ml. In the United States, the price of SH is much higher than in Japan, at $ 49.50–$ 128.00/ml, so that worldwide use of SH as a submucosal injection solution is currently impractical.

Hydroxypropyl methylcellulose (HPMC) has been identified as an economical alternative to SH that would be similarly effective at a dramatically lower cost ($ 0.15/ml), with no storage requirements and no need for reconstitution into a solution before use [19]. Hydroxypropyl methylcellulose is a cellulose derivative, with viscoelastic characteristics similar to those of SH. It is also used as an aid in eye surgery for the same purposes as SH in Western countries. The major difference in quality between SH and HPMC is that the former exists in the connective tissues of mammals and is not antigenic, whereas the latter (not available for clinical use in Japan) is a synthetic product that could potentially give rise to antigenic reactions. This is why we are unwilling to use it as an alternative to SH as a submucosal injection solution, although a recent study showed that HPMC creates long-lasting submucosal fluid cushions in the same way as SH, with minimal tissue reaction [19].

The viscoelastic measurements carried out in this study revealed two new findings on ways of increasing viscoelasticity. Firstly, doubling the molecular weight of SH can approximately double its viscoelasticity. Secondly, due to their sugar content, sugar mixtures produce a higher viscoelasticity in SH than saline solu-



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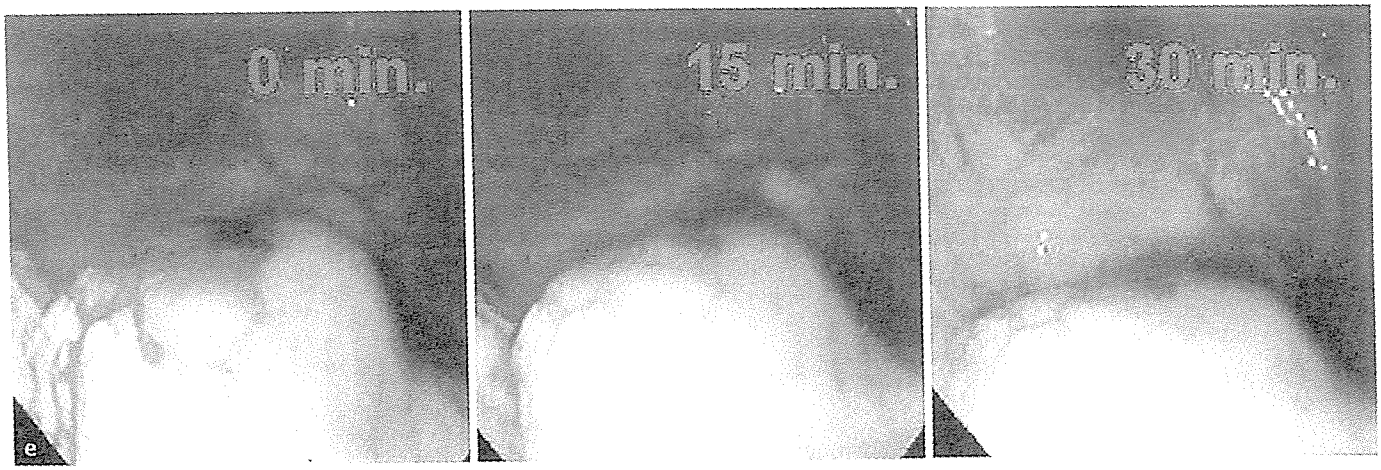

Figure 3 Endoscopic views of submucosal fluid cushions created by injecting normal saline and various mixtures of sodium hyaluronate. **a** Normal saline. **b** A 0.5% solution of 800 kDa sodium hyaluronate (SH) with normal saline. **c** A 0.25% solution of 1900 kDa SH with normal saline. **d** A 0.125% solution of 1900 kDa SH with 20% dextrose water. **e** A 0.125% solution of 1900 kDa SH with a glycerin solution (Glyceol; 10% glycerin with 0.9% saline plus 5% fructose). The mucosal elevation created by normal saline flattened out within 10 min, whereas the elevations created by the other solutions persisted at similar levels for up to 30 min, although the mucosal elevation declined over time.

tions. When SH is mixed with sugar, elastically active network chains between SH molecules can form via hydrogen bonds between SH and sugar. The molecular weight of SH in a sugar solution is therefore apparently greater than in a nonsugar solution, and the viscoelasticity of an SH solution with high sugar content may increase.

In the part of the study investigating the ability of the various solutions to create SFCs in living stomachs, similar SFCs were produced by solutions with similar levels of viscoelasticity. In addition, a 0.125% 1900 kDa SH solution made with Glyceol also created similar SFCs, which may be due to a synergistic effect of the increased viscoelasticity of SH and the hypertonic potency of glycerin. Since successful EMRs can be carried out using a 0.5% 800 kDa SH solution made with NS [17], a 0.125% 1900 kDa SH solution made with 20% DW or Glyceol, which is a low-cost solution ($ 1.25/ml), might be sufficient for successful EMR treatment.

Increasing the sugar content to more than 20% might be preferable in order to produce greater viscoelasticity. However, potential tissue damage needs to be taken into account when hypertonic solutions are used, as increased tissue damage may make it difficult to obtain a precise histological diagnosis of the resected specimens and may cause delayed ulcer healing after EMR. Extensive ulceration is often found after injection therapy for endoscopic hemostasis when 10% saline or 50% DW is used; 20% might therefore be a suitable concentration for sugar without the risk of tissue damage.

On the basis of the results presented here, the viscoelastic properties of SH can be changed using different SH molecules and different mixing solutions. Sodium hyaluronate solutions with a higher molecular weight that contain sugar with or without glycerin may allow safer EMR treatment at an acceptable cost.

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GASTROENTEROLOGY

Immunological rapid urease test using monoclonal antibody for *Helicobacter pylori*

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Abstract

Background and Aim: The current diagnostic methods for detecting *Helicobacter pylori* infection include rapid urease test (RUT), urea breath test (UBT), histology, culture, and serum antibody detection. The present study evaluated the efficacy of a novel highly specific test, an immunological RUT (IRUT), that uses a monoclonal antibody against *H. pylori* urease.

Methods: The clinical evaluation of the IRUT was performed in 100 subjects. Each gastric mucus sample obtained during endoscopic examination was incubated for 15 min with a solid tip coated with monoclonal antibody for *H. pylori* urease, and then the tip was introduced into a pH-monitoring cell containing urea solution. The change in pH of the solution after the enzymatic reaction (delta pH) was measured. The performance of the IRUT was compared with culture, histology, RUT, and UBT.

Results: Of the 47 *H. pylori*-positive subjects, 43 were IRUT positive (sensitivity, 91.5%), and of the 53 *H. pylori*-negative subjects, 52 were negative (specificity, 98.1%). Compared with the usual diagnostic methods, IRUT had high sensitivity and specificity for the detection of *H. pylori* and was no less efficient.

Conclusions: IRUT is a sensitive, specific and very rapid (within 20 min) method of detecting *H. pylori* infection.

Key words: *Helicobacter pylori*, monoclonal antibody, rapid urease test.

INTRODUCTION

Since the report of Marshall and Warren[1] there has been general agreement that *Helicobacter pylori* is closely associated with gastroduodenal disorders such as gastritis, peptic ulcer, and gastric cancer.[2] The current diagnostic methods for detecting *H. pylori* infection include rapid urease test (RUT), urea breath test (UBT), histology, culture, and serum and urinary antibody detection.[3,4] Some of those methods are based on the high urease activity of *H. pylori*,[5,6] but because they detect all enzyme activity of urease regardless of its origin, there is the possibility that urease derived from other bacterial species, such as *Proteus mirabilis* or *Klebsiella pnuemoniae*, will confound the result.

In the immunological RUT (IRUT), *H. pylori*-specific urease adsorbed onto a solid-phase tip coated with a monoclonal antibody against *H. pylori* urease is incubated with a urea solution in a urease analyzer comprising a flow-through cell for the solution, after immunological reaction with the patient's gastric mucus sample. The resulting change in pH of the solution (delta pH) is measured by ion-sensitive field-effect transistors within the cell.[7,8] We have previously used the IRUT for the detection of *H. pylori* infection[9] and the purpose of the present study was to analyze its efficacy in comparison with four conventional methods of detecting *H. pylori*; that is, RUT, UBT, histology and culture.

METHODS

Subjects

The subjects consisted of 100 inpatients who underwent thorough upper gastrointestinal endoscopy at

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Wakayama Medical University Kihoku Hospital from May 2001 to January 2002 (56 men, 44 women; mean age: 60.8 years). Of these, 21 subjects were examined after they had completed *H. pylori* eradication therapy. Those who had been prescribed proton pump inhibitors, H2 blockers, antibiotics or non-steroidal anti-inflammatory drugs prior to the examination were excluded from the study. The endoscopic diagnoses were 26 cases of non-ulcer dyspepsia, 39 cases of gastric ulcer, 20 cases of duodenal ulcer, four cases of gastric cancer, and 11 cases of gastric polyp. Informed consent was obtained from all subjects.

Sample collection

The endoscopic examination was carried out using an Olympus Videoscope (GIF-XQ230 or GIF-Q240X; Olympus Optical, Tokyo, Japan). After the routine examination, the gastric mucus sample for the IRUT was collected with a brush (163R; Olympus Optical) introduced into the stomach through the biopsy channel of the endoscope. The area in the greater curvature from the antrum to the corpus was brushed. Three biopsy specimens for culture, histology, and RUT were obtained from each of the greater curvature in the antrum and the upper corpus during the same endoscopy session. Within 14 days before or after endoscopy, tidal gas was collected for UBT as described elsewhere.[9,10]

Immunological rapid urease test

The solid-phase tips of the IRUT kit (HLS-2000; Olympus Optical) were prepared as previously described.[11,12] After its removal from the endoscope, the tip of the brush was placed in dilution buffer in a sample tube and swished vigorously. After 15 min of immunoreaction with the sample solution at room temperature, the solid-phase tip coated with a monoclonal antibody against *H. pylori* urease was introduced into the pH-measuring cell. The change in pH (delta pH) in the urea solution inside the tip after enzymatic reaction for 55 s was measured by the ion-sensitive field-effect transistors within the cell (Fig. 1).

Conventional methods of detecting *H. pylori* infection

Culture, histology, RUT and UBT were used to detect *H. pylori* infection. For culture, two biopsy samples were placed onto modified Brucella agar plates (E-MR82, Eiken Chemical, Tokyo, Japan) and incubated at 37°C for 3–5 days under microaerobic conditions. For histology, two biopsy samples were fixed in formalin and stained with Giemsa to detect *H. pylori*. The remaining two biopsy samples were used for the RUT (CLO, Ballard Medical Products, Draper, UT, USA), and were incubated for 24 h at room temperature. The UBT was performed by infrared spectrometer (UBIT-IR300, Otsuka Pharmaceutical, Tokyo, Japan).[10,11] Before the sampling of end tidal gas, the patients rinsed their mouths with water. Breath samples were collected before and 20 min after administration of 100 mL of urea solution containing 100 mg of ^{13}C-labeled urea (UBIT, Otsuka Pharmaceutical). The cut-off value of the UBT was set at 2.5 per mL. Patients with at least two positive test results from the four tests were considered as *H. pylori* positive. If only the culture was positive, the patient was also considered as infection-positive. The patients were considered to be *H. pylori* negative when the results of any three of the four tests, including culture, were negative.

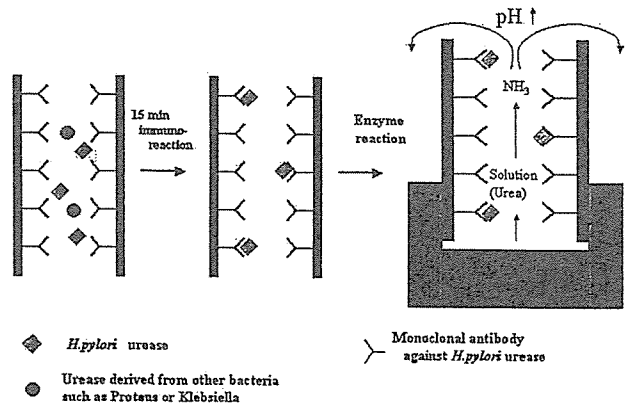

Figure 1 Schematic illustration of immunological rapid urease test. Ion-sensitive field-effect transistor (ISFET) is a pH sensor. The change in pH in the urea solution inside the tip after enzymatic reaction was measured by the ISFET.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated by standard methods. Statistical analysis was performed using the unpaired *t*-test, Pearson's correlation test, χ^2 test and Fisher's exact test. Statistical significance was indicated by $P < 0.05$.

RESULTS

In the present study, the cut-off value of the IRUT was set at delta-pH = 0.010, corresponding to 0.2 mIU/mL of *H. pylori* urease as described in a previous report.[7] According to the results of the conventional tests, there were 47 *H. pylori*-positive and 53 *H. pylori*-negative cases among the study subjects. The mean values of the delta-pH of the IRUT for *H. pylori*-positive and -negative subjects were 0.246 and 0.003, respectively ($P < 0.001$; Fig. 2). Of the 47 *H. pylori*-positive subjects, 43 were positive, and of the 53 *H. pylori*-negative subjects, 52 were negative by the IRUT, resulting in a sensitivity of 91.5% and specificity of 98.1%. The PPV and NPV for IRUT were 97.7% and 93.0%, respectively. The estimated sensitivity, specificity, PPV and

NPV of each of the conventional diagnostic methods are shown in Table 1. Compared with these widely accepted methods, the IRUT has high sensitivity and specificity for the detection of *H. pylori* and is a no less efficient diagnostic test for the bacterium. The estimated sensitivity and specificity of each of the five tests in the cases before and after eradication are shown in Table 2.

There was a significant positive correlation between the values of delta-pH of the IRUT and delta $^{13}CO_2$ of the UBT ($r = 0.39$, $P < 0.01$, data not shown). Figure 3 shows the receiver operator characteristic (ROC) curve

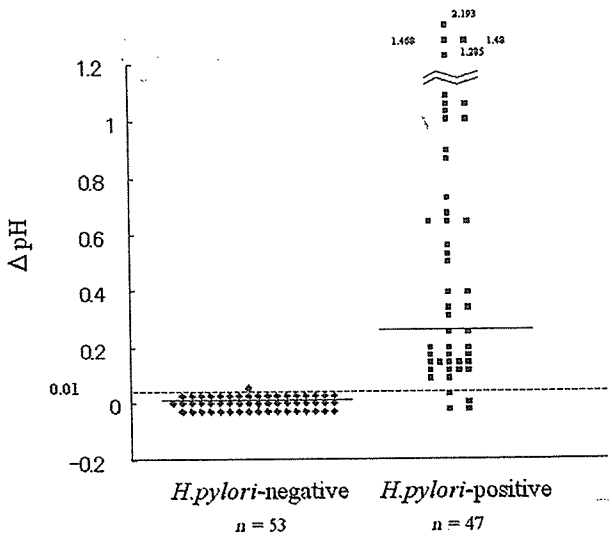

Figure 2 Distribution of delta-pH values using immunological rapid urease test. (—), mean value for each group; (---), cut-off value in the present study.

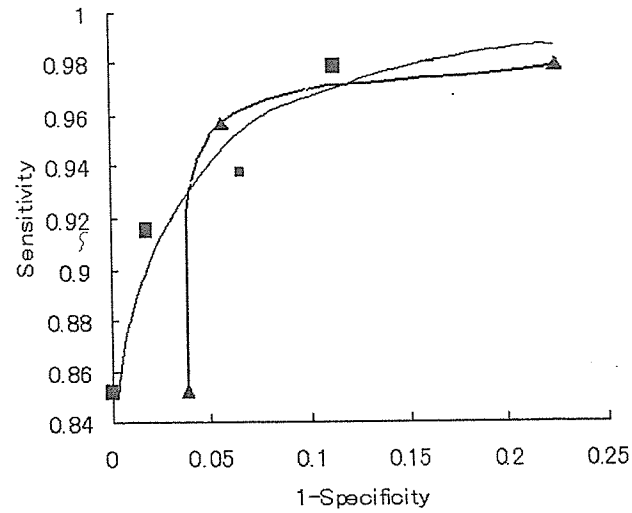

Figure 3 Receiver operating characteristic curve of the (■) immunological rapid urease test and (▲) urea breath test for the diagnosis of *Helicobacter pylori* infection.

Table 1 Efficiency of various tests for the detection of *Helicobacter pylori* (all cases: $n = 100$)

	Culture	Histology	RUT	UBT	IRUT
Sensitivity	89.4%	93.6% *	72.3% *	95.7% *	91.5%
Specificity	100% *	88.7% *	100% *	94.3% *	98.1%
Positive predictive value	100% *	88.0% *	100%	93.8%	97.7%
Negative predictive value	91.4%	94.0%	80.3%	90.4%	93.0%

RUT, rapid urease test; UBT, urea breath test; IRUT, immunological RUT. *$P < 0.05$.

Table 2 Efficiency of various tests for the detection of *Helicobacter pylori* before and after eradication

	Culture	Histology	RUT	UBT	IRUT
Cases before eradication: $n = 79$					
Sensitivity	90.9%	93.2% *	72.7% *	95.5%	90.9%
Specificity	100% *	91.4% *	100% *	94.3%	100%
Cases after eradication: $n = 21$					
Sensitivity	66.7%	100%	66.7%	100%	100%
Specificity	100%	83.3%	100%	94.4%	94.4%

RUT, rapid urease test; UBT, urea breath test; IRUT, immunological RUT. *$P < 0.05$.

of the IRUT and UBT, which revealed a considerable overlap; but it clearly indicates that the diagnostic ability of the IRUT is at least comparable or even superior to that of the UBT.

No significant adverse effects of the IRUT were observed throughout the study.

DISCUSSION

There is general agreement that *H. pylori* infection is closely associated with chronic gastritic conditions, including intestinal metaplasia, peptic ulcer and gastric cancer. Several methods for detecting *H. pylori* infection have been developed and of them the IRUT is simple, easy and aims to achieve more sensitive and rapid detection.[13–15] It takes conventional RUT a relatively long time (usually between 1 h and 24 h) to detect *H. pylori* infection, but an IRUT can detect it within 20 min. In addition, the sensitivity of IRUT is significantly higher than RUT, and the specificity is significantly higher than histology (Table 1); in the present study, the sensitivity, specificity, PPV and NPV for the IRUT were 91.5%, 98.1, 97.7% and 93.0%, respectively, whereas the RUT had a sensitivity of 72.3%, which is relatively low compared with previous reports (88–95%). Our results may be related to the patchy distribution of *H. pylori* in the stomach or to the inclusion of a considerable number of subjects who either had undergone eradication therapy (21%) or who were aged (mean age, 60.8 years). The sensitivity and specificity of the IRUT used in the present study were comparable to those for UBT, which is based on non-specific urease activity, regardless of the bacterial species. In both tests the enzyme activity of urease is measured after biochemical reaction, so it is quite reasonable that there was good correlation between the results of the two tests. However, the UBT detects all urease activity, whereas the IRUT specifically detects *H. pylori*-derived urease. The ROC analysis revealed that the two curves overlap considerably and that the two tests are almost equally reliable. Furthermore, the IRUT is less expensive than the UBT (¥600 vs ¥3357 for reagent and ¥980 000 vs¥1870 000 for apparatus, respectively) and the results can be obtained more rapidly. The UBT has been widely used as a test after *H. pylori* eradication therapy to judge whether the therapy has been successful,[6,16] and the high sensitivity and specificity of the IRUT indicates that it would also be useful for this task (Table 2). The observed high sensitivity of the IRUT is probably related to the method of collecting the sample and the high specificity is based on the use of monoclonal antibody against *H. pylori*. A major factor in a false-negative diagnosis of *H. pylori* infection is the patchy distribution of both the bacteria and intestinal metaplasia within the gastric mucosa,[17,18] and it is now considered reasonable to analyze gastric mucus rather than a mucosal biopsy specimen.[19] We have confirmed that when broad gastric brushings are used to collect the mucus sample, a larger area of the gastric surface can be sampled, giving greater reliability even with a small sample size, which ultimately leads to sensitivity comparable to those by histology or UBT, described here.

In conclusion, the IRUT enables sensitive, specific and very rapid (within 20 min) detection of *H. pylori* infection.

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