a general health checkup (baseline). Aliquots of the separated sera were stored below −20°C until measurement. In Japan, these health checkup programs are carried out to find incident diseases in the early stages. Therefore, subjects who had specific symptoms were excluded. Symptom-free subjects took part in the following tests and procedures: an interview to ascertain general state of health, physical examination, chest X-ray, electrocardiogram, blood laboratory tests, urinalysis and fecal occult blood test. Since the health checkup program was targeted at middle-aged employees, the age distribution of the subjects was relatively limited. Women were excluded from the study because of their small number ($n=65$).

Surveillance method

The subjects were screened annually to identify incident gastric cancer cases during the 8-year period between 1994 and 2002. Surveys for gastric cancer were conducted using a combination of screening methods, as follows: All subjects were screened by double-contrast barium X-ray using computer radiography. Those with positive X-ray findings and/or a positive PG test were further examined by panendoscopy (Types XQ200, Olympus, Tokyo, Japan). The histopathological assessment was done on a resected specimen obtained by endoscopy or surgery. Early gastric cancers were defined as those confined to the mucosa or submucosa and advanced cancers as those invading the muscularis propria or beyond. Pathologically, gastric cancer cases were classified as intestinal type or diffuse type, according to Lauren's classification.[36] We regarded the incident day of gastric cancer as the day of the health checkup when the cancer was detected. The length of the observation period was calculated for each subject from the time of the baseline survey to that of the diagnosis of gastric cancer. The ethics committee of Wakayama Medical University approved the protocol and informed consent was obtained from all participating subjects.

Serologic analysis

PG levels were measured by PG I/PG II RIA-Bead Kits (Dainabbot Co., Ltd., Tokyo, Japan), a modified method of the radioimmunoassay, which we have previously established.[37] Subjects with extensive CAG were diagnosed on the basis of the previously described PG test positive criteria (i.e., PG I<70 μg per liter and PG I/II<3.0).[38-40] High sensitivity (70.5%) and specificity (97%) for these criteria in the diagnosis of extensive CAG has been reported and warrants the validity of the criteria.[39] Anti-H. pylori IgG antibody levels were measured by ELISA (MBL, Inc., Nagoya). Subjects with an anti-H. pylori IgG antibody titer of more than 50 U per milliliter were classified as H. pylori-infected. Those that were negative or had less than 30 U per milliliter were regarded as infection negative. Subjects between 30 U per milliliter and 50 U per milliliter were considered indeterminate and were excluded from the study. The sensitivity and specificity for the ELISA used in the present study was 93.5% and 92.5%, respectively.[41]

Statistical analysis

Data were analyzed by SPSS (SPSS, Inc., Chicago, Illinois, USA) and STATA (STATA Corp., College Station, TX). Differ-

ences were tested for significance using t-test for the comparison of 2 groups, analysis of variance (ANOVA) for the comparison among multiple groups and Scheffe's LSD test for pairs of groups. For comparison of categorical variables, chi-square test was used. We evaluated the long-term effects of CAG and H. pylori infection on the incidence of gastric cancer using Cox proportional-hazards models.

RESULTS

Among the 5,706 subjects who were eligible, a total of 1,059 declined to participate, had previously undergone gastric resection, or had been prescribed proton pump inhibitors, H2 blockers, or nonsteroidal anti-inflammatory drugs prior to the examination. These subjects were excluded from the study. Eight cases of gastric cancer diagnosed within the first year of surveillance were also excluded from the analysis. The remaining eligible 4,655 subjects, including 45 incident gastric cancer cases detected during the study period, were analyzed.

The baseline characteristics of all subjects and the subjects classified by H. pylori infection or CAG are shown in Table I. The mean age±standard deviation of all 4,655 subjects at the time of the initial survey was 49.5±4.6 years old and the mean follow-up period was 7.7±0.9 years. The mean values of serum PG I and PG I/II ratio were 61.2±30.4 μg per liter and 4.1±2.1, respectively. Using the 2 serum tests (anti-H pylori antibody and PG level), we evaluated H. pylori infection and CAG in the study population and found that 78.6% (3,657/4,655) of the subjects were infected with H. pylori and 28.9% (1,347/4,655) were CAG-PG test positive. The mean age of the subjects in the H. pylori-positive or CAG-positive groups was significantly higher than in the respective negative groups. The duration of follow-up in the H. pylori-positive or PG-positive groups was significantly shorter than that in the respective negative groups. In the H. pylori-negative group, CAG was positive in 3.1% (31/988) of the subjects, whereas in the H. pylori-positive group 36.0% (1,316/3,657) of the subjects were positive for CAG. H. pylori infection was diagnosed in 70.8% (2,341/3,308) of CAG-negative subjects and in 97.7% (1,316/1,347) of CAG-positive subjects. The observed differences were all significant. The serum PG I level and the I/II ratio were also significantly different between the groups with and without H. pylori infection or CAG ($p<0.001$).

Table II shows the development of gastric cancer among all subjects and among H. pylori-negative/positive or CAG-negative/positive subjects. As mentioned above, 45 gastric cancer cases were detected during 35,708 person-years of follow-up, putting the incidence rate of gastric cancer in the cohort at 126/100,000 person-years. The mean age of all the gastric cancer subjects was 51.5±3.9 years and the mean follow-up time was 4.9±2.0 years. Among the 45 incident cancers, 43 (95.6%) developed in the H. pylori-positive group, and 26 (57.8%) in the CAG-positive group. The incidence rates of gastric cancer in H. pylori-positive and H. pylori-negative groups were 154/100,000 and 26/100,000 person-years, respectively. The incidence rates for those in CAG-positive and CAG-negative groups were 255/100,000 and 74/100,000 person-years, respectively. The mean age and mean follow-up periods

TABLE I—BASELINE CHARACTERISTICS OF THE SUBJECTS[1]

	Total	H. pylori		CAG	
		Negative	Positive	Negative	Positive
Subjects (n)	4,655	998	3,657	3,308	1,347
H. pylori positive	3,657	—	—	2,341	1,316
CAG positive	1,347	31	1,316	—	—
Age (years)	49.5 (4.6)	48.3 (4.5)	$49.8 (4.6)^2$	49.1 (4.6)	$50.4 (4.3)^2$
Duration of follow-up (years)	7.7 (0.9)	7.7 (0.8)	$7.6 (0.9)^2$	7.7 (0.8)	$7.6 (1.0)^2$
PGI (μg/l)	61.2 (30.4)	58.2 (20.7)	$62.0 (32.5)^2$	70.9 (29.1)	$37.3 (17.5)^2$
PGI/II	4.1 (2.1)	6.4 (1.7)	$3.4 (1.6)^2$	4.9 (1.8)	$1.9 (0.7)^2$

[1]Mean (SD).–[2]Significantly different from the respective negative group ($p < 0.001$).

TABLE II – THE DEVELOPMENT OF GASTRIC CANCER[1]

	Total	H. pylori		CAG	
		Negative	Positive	Negative	Positive
Cancer cases	45	2	43	19	26
H. pylori positive cases	43	—	—	19	24
CAG positive cases	26	2	24	—	—
Age (years)	51.5 (3.9)	54.0 (0.0)	51.4 (3.9)	51.2 (4.5)	51.8 (3.5)
Follow-up years (years)	4.9 (2.0)	3.8 (3.4)	5.0 (2.0)	5.3 (2.1)	4.6 (2.0)
Incidence rate[2]	126	26	154	74	255
Histopathological type					
Intestinal type (cases/incidence rate)[2]	30/84	1/13	29/104[3]	11/43	19/186[4]
Diffuse type (cases/incidence rate)[2]	15/42	1/13	14/50	8/31	7/69
Stage of progress					
Early stage (cases/incidence rate)[2]	41/115	2/26	39/140	17/67	24/235
Advanced stage (cases/incidence rate)[2]	4/11	0/0	4/14	3/12	1/10

[1]Mean (SD).–[2]Per 100,000 person-year.–[3]Significantly different from *H. pylori* negative group ($p < 0.05$).–[4]Significantly different from CAG negative group ($p < 0.05$).

TABLE III – INCIDENCE RATE AND HAZARD RATIO (HR) OF GASTRIC CANCER AMONG THE GROUPS CATEGORIZED
BY *H. PYLORI* INFECTION AND CAG (ASSIGNED 1 CASE TO GROUP A)[1]

Group	A	B	C	D	
H. pylori infection	–	+	+	–	*p* (trend)
CAG	–	–	+	+	
Subjects	967	2341	1316	31	
Person-years	7568	17835	10074	230	
Age (years)	48.3 (4.5)	49.5 (4.7)[3]	50.4 (4.3)[3,4]	49.4 (4.79)	
Follow-up years (years)	7.83 (0.61)	7.67 (0.85)	7.58 (0.97)[3,4]	7.41 (1.47)	
PGI (μg/l)	59.4 (19.7)	75.8 (31.1)[3]	37.9 (17.5)[3,4]	22.2 (19.1)[3,4,5]	
PGI/II	6.57 (1.54)	4.26 (1.41)[3]	1.96 (0.66)[3,4]	1.68 (0.84)[3,4]	
Total gastric cancer					
cases/incidence rate[2]	0/–(1/13)[6]	19/107	24/238	2/871	
HR (95% CI)	–(1)[6]	7.13 (0.95–53.33)	14.51 (1.96–107.70)	61.85 (5.60–682.64)	0.0007
Intestinal gastric cancer					
cases/incidence rate[2]	0/–(1/13)[6]	11/62	18/179	1/435	
HR (95% CI)	–(1)[6]	4.07 (0.52–31.57)	10.65 (1.41–80.26)	30.38 (1.90–486.22)	0.0065
Diffuse gastric cancer					
cases/incidence rate[2]	0/–(1/13)[6]	8/45	6/60	1/435	
HR (95% CI)	–(1)[6]	3.02 (0.37–24.27)	3.65 (0.44–30.65)	31.77 (1.99–508.59)	0.0852

[1]Mean (SD).–[2]Per 100,000 person-year–[3]Significantly different from group A ($p < 0.05$).–[4]Significantly different from group B ($p < 0.05$).–[5]Significantly different from group C ($p < 0.05$).–[6]In reality-the cancer incidence in group A is null and the comparison of the cancer risk among the group was impossible. Therefore, we tentatively presume that a single cancer cases derived from group A during the study period of 8 years (incidence rate of 13/100,000 person-year). The adjusted HR was calculated in each group according to Cox proportional-hazards model.

were not significantly different among these subgroups: *H. pylori*-positive and *H. pylori*-negative, or CAG-positive and CAG-negative. In a univariate analysis after adjustment for age, *H. pylori* infection was associated with a significantly increased risk of gastric cancer [hazard ratio, 5.13 (95% confidence interval, 1.24 to 21.24) $p<0.05$]. A positive PG test reflecting coexisting CAG was also associated with a significantly increased risk of gastric cancer [hazard ratio, 3.03 (95% confidence interval, 1.67 to 5.49) $p<0.001$].

Histopathological analysis of gastric cancer detected in the present study revealed that 30 cases (67%) were intestinal type and the remaining 15 cases (33%) were diffuse type (Table II). Forty-one cases (91%) were in the early stage and the remaining 4 cases (9%) were in the advanced stage. Seventy-six percent of the early cancer cases were limited to the mucosal layer. Thus, pathologists in some Western countries would be more likely to classify some of these lesions as high-grade adenoma/dysplasia.[42] A majority of the cancer cases in each of the 2 hisotopathological types (intestinal type 96.7% and diffuse type 93.3%) developed in the anti-*H. pylori* antibody-positive group. Also, the incidence rate was considerably higher in the positive group (intestinal type 104/100,000 and diffuse type 50/100,000) compared to the negative group (intestinal type 13/100,000 and diffuse type 13/100,000). There was a significant difference, however, only for the intestinal-type cancer. Likewise, 63.3% of intestinal-type cancers and 46.7% of

diffuse-type cancers developed in subjects with a positive PG test. Similarly, a significant difference in the incidence rates among these subjects was found only in those with intestinal-type cancer.

In the next study, the relation between the progression of *H. pylori*-induced CAG and the development of gastric cancer was investigated. Study subjects were placed in 1 of 4 groups based on the results of the 2 serologic tests, anti-*H. pylori* antibody titer, and serum PG. The 4 groups were (1) group A for *H. pylori*(−)/ CAG(−) subjects, (2) group B for *H. pylori*(+)/CAG(−) subjects, (3) group C for *H. pylori*(+)/CAG(+) subjects and (4) group D for *H. pylori*(−)/CAG(+) subjects. We analyzed the development of gastric cancer among the 4 groups. The baseline characteristics of each group are shown in Table III. The mean age increased from group A to C but declined in group D. Group A had the longest follow-up period and the highest PG I/II ratio. These values decreased in a stepwise manner from A to D. Serum PG I level was the highest in group B, followed by groups A, C and D. These differences among the 4 groups were all significant ($p<0.0001$).

Figure 1 shows the Kaplan-Meier analysis of the subjects classified by the above-mentioned subgroups. After 2 years of observation, the percentage of subjects free from gastric cancer was highest in group A, followed by B, C and D. The incidence rate and hazard ratio of gastric cancer in each group are also shown in Table III. The incidence rate of gastric cancer was null (0/967),

107/100,000 person-years (19/2,341), 238/100,000 person-years (24/1,316), and 871/100,000 person-years (2/31) for groups A, B, C and D, respectively. Since the cancer incidence in group A was null, making comparison of the cancer risk among the groups impossible, we assumed that a single cancer case occurred in group A during the 8-year study period (incidence rate 13/100,000 person-years). The adjusted hazard ratio was calculated in each group according to the Cox proportional hazards model. As a result, there was a stepwise increase in the adjusted hazard ratio for gastric cancer among the groups from A to D, reaching the highest ratio of 61.85 in group D. The difference between group A and B was not significant. The same stepwise increase was observed in the incidence rate of intestinal-type cancer. The hazard ratio that was calculated based on the same assumption also showed the same significant stepwise increase among the groups, except between groups A and B. This trend was also observed in the diffuse-type cancer. However, the increase in the hazard ratio was significant only in group D.
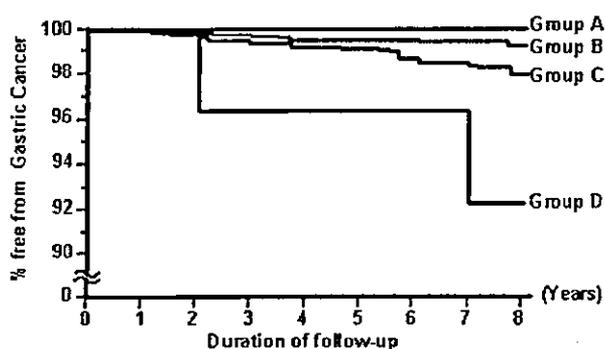

FIGURE 1 – Kaplan-Meier analysis in relation to the progression of chronic atrophic gastritis (CAG). Subjects were classified into 1 of 4 groups (A to D) based on the results of the 2 serologic tests, anti-*H. pylori* titer and serum pepsinogen level. Group A [*H. pylori*(−)/ CAG(−)], infection free subjects; Group B [*H. pylori*(+)/CAG(−)], CAG-free gastritis; Group C [*H. pylori*(+)/CAG(+)], CAG and Group D [*H. pylori*(−)/CAG(+)], severe CAG with extensive intestinal metaplasia. In group A, no cancer developed during the study period and the incidence rate was null. The cancer incidence rate for groups B, C and D was 107/100,000 person-years, 238/100,000 person-years, and 871/100,000 person-years, respectively.

Since the number of subjects and the incident number of cancer cases in group D were small, we examined the relationship between the risk of gastric cancer and the extent of CAG using another type of analysis. Our previous analysis indicated that PG I/II ratio and gastric secretory function decreased progressively as the functional fundic gland got smaller during the course of CAG progression.[34] We also found that the PG I/II ratio gives a precise measure for the extent of CAG. Therefore, the study subjects were classified into 3 groups according to PG I/II ratio. The 3 groups were (1) group X for a ratio greater than or equal to 3.0, (2) group Z for a ratio less than 2.0 and (3) group Y for a ratio between X and Z. The baseline characteristics of each group are shown in Table IV. The mean follow-up period tended to decrease from group X to group Z. There was a stepwise increase in the mean age from group X to group Z, while the serum level of PG I decreased in a stepwise fashion from group X to group Z. The incidence rate and hazard ratio for gastric cancer in each group is shown in Table IV. The incidence rate of gastric cancer increased in a stepwise manner as the PG I/II ratio decreased: 77/100,000 person–years in group X to 250/100,000 person–years in group Z, leading to a significant stepwise increase in the adjusted hazard ratio from group X to group Z. The same significant stepwise increase in the incidence rate and hazard ratio with reduction in the I/II ratio was also observed in the intestinal-type cancer, whereas no such change was observed in the diffuse-type cancer.

DISCUSSION

Previous epidemiological studies have indicated an association between *H. pylori* infection and gastric cancer.[9-18] In addition, clinicopathological evidence has shown that the progression of atrophic gastritis increases the risk of gastric cancer,[1,27] but this area requires more long-term studies. Up to now, there have been few prospective studies that have investigated the relationship between the extent of CAG and the development of gastric cancer in hospital patients with atrophic gastritis.[28-30] The results of these studies are conflicting. One study indicates that incomplete and unstable CAG is directly associated with development of cancer,[28] whereas the others show a positive correlation between the extent of CAG and cancer development.[29,30] To approach these problems, we evaluated the risk of gastric cancer in a cohort of 4,655 asymptomatic healthy male subjects after a mean follow-up period of 7.7 years. During the follow-up period, 45 gastric cancers were detected at an incidence rate of 126/100,000 person-years. The incidence rate in our study is low compared to those presented by the other prospective studies (217.5 to 223.1/100,000 person-

TABLE IV – INCIDENCE RATE AND HAZARD RATIO (HR) OF GASTRIC CANCER BETWEEN SUBGROUPS DEFINED BY SERUM PG I/II RATIO[1]

Group PG I/II	X ≥3	Y 2–3	Z <2	p (trend)
Subjects	3,043	920	692	
Person-years	23,510	6,997	5,200	
Age (years)	49.0 (4.6)	50.0 (4.5)[3]	50.1 (4.2)[3,4]	
Follow-up years (years)	7.71 (0.77)	7.61 (0.94)[3]	7.51 (1.01)[3,4]	
H. pylori infection	2079	906	672	
CAG	59	646	642	
PGI (μg/l)	68.0 (28.2)	60.1 (29.3)[3]	32.8 (24.3)[3,4]	
PGI/II	5.16 (1.68)	2.45 (0.28)[3]	1.38 (0.40)[3,4]	
Total gastric cancer cases/incidence rate[2]	18/77	14/200	13/250	
HR (95% CI)	1	2.39 (1.19–4.82)	2.75 (1.34–5.65)	0.009
Intestinal gastric cancer cases/incidence rate[2]	11/47	9/129	10/192	
HR (95% CI)	1	2.5 (1.03–6.05)	3.43 (1.44–8.12)	0.01
Diffuse gastric cancer cases/incidence rate[2]	7/30	5/72	3/58	
HR (95% CI)	1	2.23 (0.70–7.05)	1.67 (0.43–6.53)	0.4

[1]Mean (SD).–[2]Per 100,000 person-year.–[3]Significantly different from group X (p < 0.05).–[4]Significantly different from group Y (p < 0.05).

years) probably because the other studies are all based on hospitalized patients.[28,29] According to cancer statistics from 1997, the age-adjusted incidence rate of gastric cancer among Japanese men between the ages of 40 and 60 was 95.7/100,000 person-years.[3] This ranged from a low of 35.0/100,000 person-years for 40- to 44-year-old men to a high of 182.4/100,000 person-years for 55- to 59-year-old men. Our higher rates are probably partly due to our use of the sensitive screening system of double-contrast barium X-ray using computer radiography combined with endoscopy and partly because our study region is in a high-risk gastric cancer area. The gastric cancer mortality rate in this district ranked 7th out of 47 administrative districts in Japan during the same year.[3]

Consistent with the reported elevated prevalence of *H. pylori* infection or CAG in the high-risk cancer population,[1,4,17] there was a high prevalence of positive specific antibody titer (78%) and CAG-PG positive tests (28.9%). Indeed, based on univariate analysis, both factors, *H. pylori* infection and CAG, were associated with a significantly increased risk for gastric cancer.

To clarify the influence on risk of gastric cancer by the interaction of *H. pylori* infection and CAG, we divided the population consisting of healthy asymptomatic subjects into 4 groups. The groups were determined by the results of the 2 serologic tests (PG and anti-*H. pylori* antibody). This classification reflects each stage of the serial changes in stomach mucosa induced by chronic *H. pylori* infection. The *H. pylori*-free healthy condition corresponds to 2 negative tests (group A). With the establishment of *H. pylori* infection, the antibody test becomes positive (group B). As the infection spreads, the PG test also turns positive (group C). Intestinal metaplasia develops and spreads in the presence of CAG, leading to reduction of the bacterial load in the stomach.[13,43,44] This results in a negative specific antibody test (group D). Thus, group D comprises those subjects with metaplastic gastritis. Indeed, the serum PG level was the highest in group B and decreased in a stepwise manner from B to D. Endoscopic findings from each of the 4 groups also confirmed the above-mentioned changes of the stomach mucosa from A to D. Among the 45 incident gastric cancers, only 2 cases (4.4%) were *H. pylori*-negative. However, based on the above classification, these *H. pylori*-negative cancers belong in group D. The observed seronegativity of the cancers appears to be the end result of *H. pylori* infection. Therefore, it can be concluded that all the incident cancers in the present study are *H. pylori*-infection positive and no cancer arose in subjects with healthy stomach mucosa (group A). With the progression of *H. pylori*-induced gastritis, we observed a stepwise significant increase in the incidence rate and the hazard ratio for total gastric cancer, indicating a positive correlation between the extent of CAG and cancer development.

Group D, comprising 0.7% of the cohort, was at highest risk for gastric cancer. However, the number of subjects in group D (n=31) and the number of gastric cancer cases in the group (n=2) were relatively small. We therefore used another type of analysis to estimate the relation between the development of cancer and the progression of CAG. Serum PG levels, especially the PG I/II ratio, are powerful markers for gastric atrophy. Previous studies clearly indicate the PG I/II ratio is almost as effective as the maximal acid output value in the detection of extensive atrophy.[34] The ratio is also more reliable than PG I.[34] Therefore, the study subjects were divided into 3 groups based on the PG I/II ratio, and the risk of the cancer was analyzed in these groups. Our analysis showed that the cancer was more frequent and the hazard ratio was significantly higher in the group with a lower PG I/II ratio. This confirmed a dose-response correlation between cancer development and the

progression of atrophic gastritis. These results are in accordance with various clinicopathological and epidemiological studies indicating an association between cancer development and extensive atrophic gastritis, especially intestinal metaplasia.[1,4,27] Our results clearly indicate that *H. pylori* infection is a common risk factor for the both intestinal- and diffuse-type cancer, but the association is stronger for the intestinal type. Additionally, only intestinal-type cancer had an increased risk during the progression of gastritis, strongly supporting the hypothesis that there is a difference in the mechanism of carcinogenesis for the 2 types of cancer. The diffuse-type cancer has no accepted model for carcinogenesis. However, the hypothesis that *H. pylori* infection precedes and plays a pivotal role in the outcome of the cancer is supported by findings on rodents.[24-26] These experiments show that the introduction of *H. pylori* infection causes an increase in the development of this type of cancer with dramatically higher efficiency compared to the conventional experimental method.

The current model for the development of intestinal-type cancer begins with gastritis induced by *H. pylori*. During the course of chronic inflammation, altered gene expression occurs.[45] In addition, the structure and function of the genes may change and accumulate.[46,47] As a result of these molecular events, a series of changes in the histological structure of the stomach mucosa occur, thus following each step of the extensive atrophic gastritis-metaplasia-dysplasia-carcinoma sequence.[27] As described above, the progression of atrophic gastritis tends to make *H. pylori* less prevalent but still leads to a steady growth in cancer development. Thus, it is quite probable that the bacterium itself is necessary but not sufficient for the development of the cancer. Rather, the end result of chronic inflammation caused by the bacterial colonization is more important than *H. pylori* infection itself for the development of cancer, especially the intestinal type. Since the observed incidence rate of gastric cancer in subjects with *H. pylori* infection is comparatively low, even in group D, other environmental or genetic cofactors are probably involved in the progression to cancer.

The results of the present study have also made it clear that the 2 serum tests allow identification of individuals who are at especially high risk for gastric cancer (group D). Fortunately, according to our investigations in several other areas, individuals with test results the same as subjects in group D constitute less than 1% of the total population (M. Ichinose, unpublished data). It is advisable that this small number of subjects should have regular, detailed endoscopic examinations. In addition, these results will contribute to a more efficient cancer screening system by making it possible to exclude a group of individuals at low risk for gastric cancer (group A), which accounts for nearly 20% of the target population.

Finally, the results of the present study confirm the previously reported data that both *H. pylori* infection and *H. pylori*-induced CAG have an important role in the development of gastric cancer. The data clearly demonstrate that it is quite rare for gastric cancer to develop in the *H. pylori*-free stomach, regardless of the histopathological type. In addition, the progression of CAG dramatically increases the risk of cancer, especially the intestinal type, with a clear dose-response relationship. Although involvement of other unknown cofactors in stomach carcinogenesis is strongly implied, eradication of the bacterium will probably be important in reducing the risk of *H. pylori*-related carcinogenesis by preventing the progression of CAG to the final stage of the infection, metaplastic gastritis.

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Figure 1 A healthy 59-year-old man underwent colonoscopy because of a positive fecal blood test. A yellowish-white lobulated polyp, 15-mm in size with a "baby's hand"-like morphology, was seen. Endoscopic polypectomy for suspected leiomyoma was performed without complication.
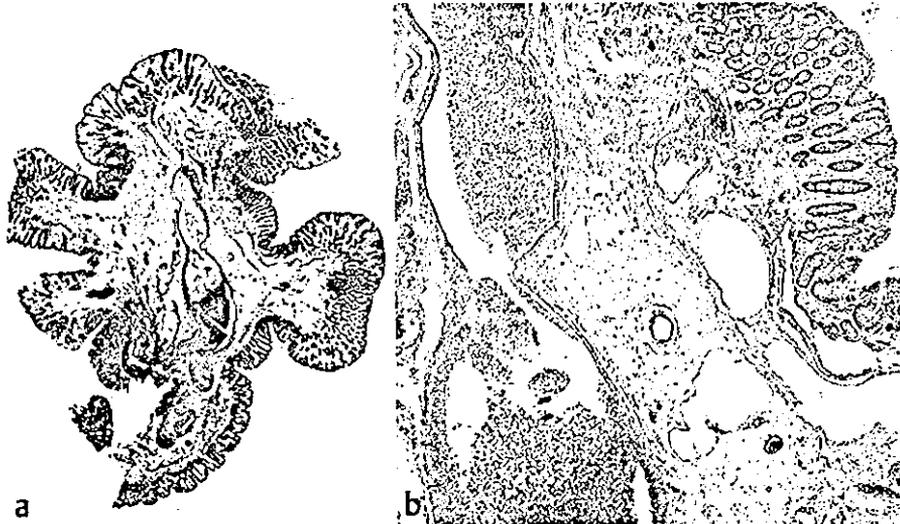

Figure 2 The edematous stroma was filled with enlarged serpiginous veins and arterioles, leading to a diagnosis of angiodysplasia. Angiodysplasia is one of the major causes of lower gastrointestinal bleeding, often encountered during emergency colonoscopy. The typical endoscopic appearance is often reported to be a slightly elevated reddish lesion, reflecting the dilated, tortuous veins in the submucosa. Lesions having a polypoid morphology are extremely rare. This case suggests us the possibility of angiodysplasia as a polypoid lesion, and the need for care when performing biopsy or endoscopic polypectomy.

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NEW INSTRUMENTS AND TECHNIQUES

ENDOSCOPIC SUBMUCOSAL DISSECTION FOR EARLY GASTRIC CANCER USING THE TIP OF AN ELECTROSURGICAL SNARE (THIN TYPE)

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Background: Although the strip biopsy method and aspiration method are popular endoscopic mucosal resection techniques for its convenience and reliability, they have limitations in resectable tumor size and location. Endoscopic submucosal dissection techniques using the diathermic needle knife or the insulated-tip diathermic knife have been introduced to overcome this disadvantage, but they have high risks for bleeding and perforation. Therefore, we have developed a new endoscopic submucosal dissection technique using the tip of an electrosurgical snare (thin type) and assessed its efficacy.
Methods: Fifty-nine lesions with differentiated-type gastric cancer without ulceration were treated with our technique at the University Hospital. The tip of an electrosurgical snare (thin type) was used for mucosal incision and submucosal dissection as a flexible diathermic knife.
Results: The size of tumor was 5–85 mm in diameter (mean size: 29 mm) and the location varied from cardia to antrum. Among 59 lesions, 56 lesions (56/59, 95%) were resected completely in an en-bloc fashion with much less perforation (2/59, 3.4%) and bleeding (1/59, 1.7%) regardless of their size and location.
Conclusion: New endoscopic submucosal dissection technique using the tip of an electrosurgical snare (thin type) is safe and reliable. We were able to resect early gastric cancer with a much higher en-bloc resection rate and fewer complications using this technique.

Key words: electrosurgical snare (thin type), en-bloc resection, endoscopic mucosal resection, endoscopic submucosal dissection, tip of snare.

INTRODUCTION

Many endoscopic mucosal resection (EMR) techniques have been developed in Japan for the treatment of gastric mucosal lesions. In these techniques, strip biopsy[1] and aspiration mucosectomy using an attached hood on the tip of endoscope[2,3] have become popular practice for its convenience and reliability. However, the specimens obtained by these techniques have limitations in size (approximately 10–20 mm, according to tumor location and operators' skills) and it often becomes piecemeal resection in larger tumors. En-bloc resection is desirable in especially larger tumors, because histological evaluations are essential to estimate the risk for lymph node metastasis. Moreover, it is also very important for prevention of local recurrence, as a considerably high recurrence rate after piecemeal resection has been reported.[4] Endoscopic submucosal dissection techniques using the diathermic needle

knife[5] or the insulated-tip diathermic knife (IT knife)[6,7] have been introduced to overcome this disadvantage, but different problems have emerged: the complication rates (i.e. 22% of bleeding, 5% of perforation using the IT knife)[7] are relatively high. Therefore, we have developed a new endoscopic submucosal dissection technique using the tip of an electrosurgical snare (thin type; SD-7P-1, Olympus, Tokyo, Japan) (Fig. 1) as a flexible diathermic knife and assessed the efficacy and complications of the new technique.

PATIENTS AND METHODS

Between February 2000 and June 2002, 59 lesions were treated with the new technique at the university hospital, University of Tokyo, Japan. Candidates for endoscopic treatment were determined by endoscopic features with chromoendoscopy, endoscopic ultrasonography (EUS) and endoscopic biopsy. The following criteria were met by the subjects of EMR: (i) intramucosal tumor diagnosed by endoscopy and EUS; (ii) no endoscopically apparent ulceration or fold convergence; and (iii) a histological diagnosis of differentiated-type adenocarcinoma from biopsy.
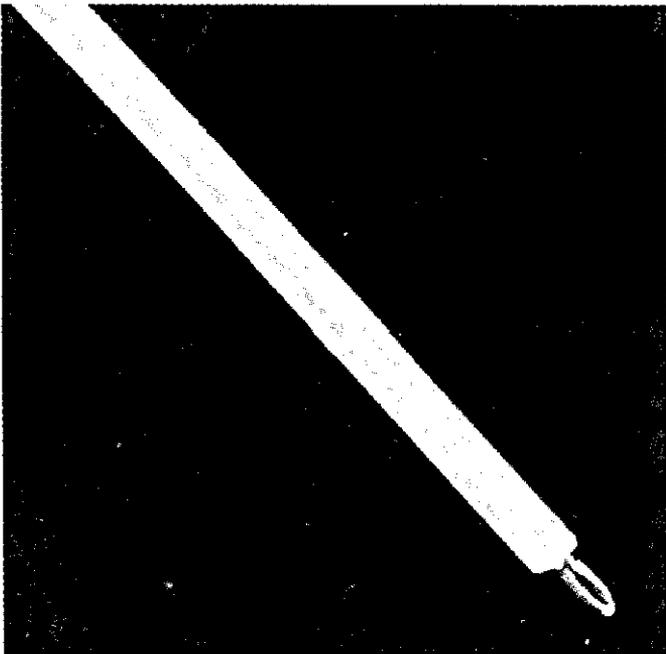
Fig. 1. An electrosurgical snare (thin type, SD-7P-1; Olympus). The length of the snare is adjustable according to the situation. For marking dots, 0.5–1 mm is long enough, but for mucosal incision and submucosal dissection 1–2 mm is the appropriate length.

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
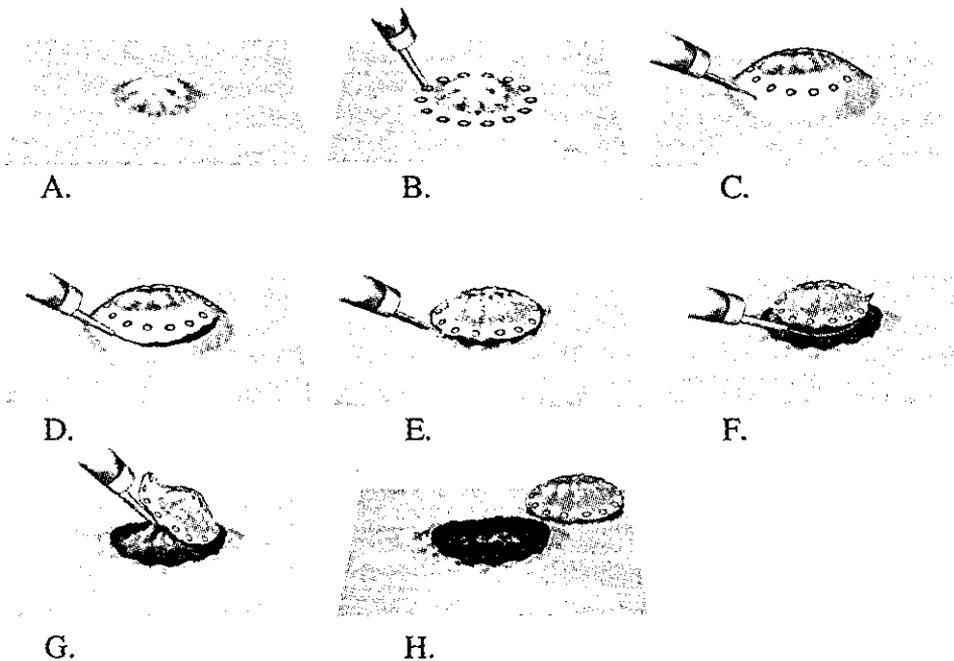

Fig. 2. Schematic drawing of the endoscopic mucosal resection (EMR) procedure using an electrosurgical snare (thin type). A, small elevated lesion without ulceration. B, marking dots are made using the tip of an electrosurgical snare (thin type) on the circumference of the lesion. C, 20% glucose solution (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. Perfo-

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.

ration 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.

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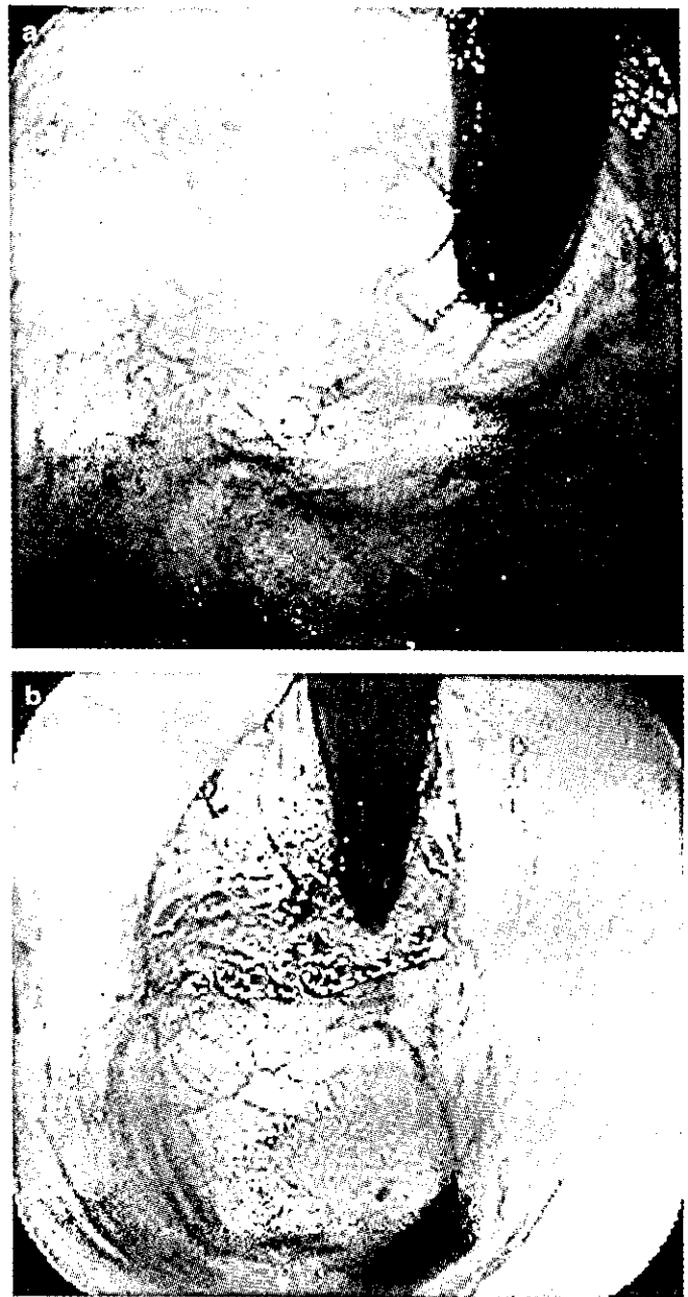
Fig. 4. A large IIa lesion located in a difficult area for endoscopic treatment. The lesion is distributed from the esophagogastric junction to cardia. The lesion was resected completely en bloc, despite its difficult location.

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 prob-

lems 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.

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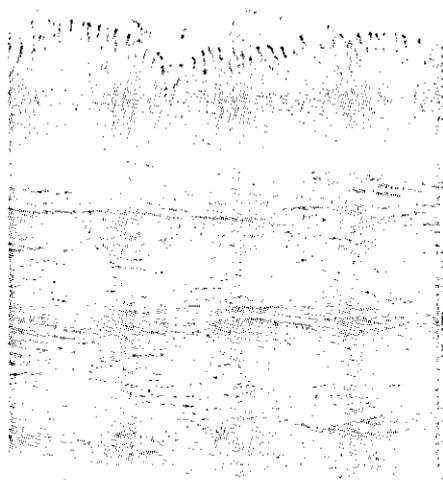
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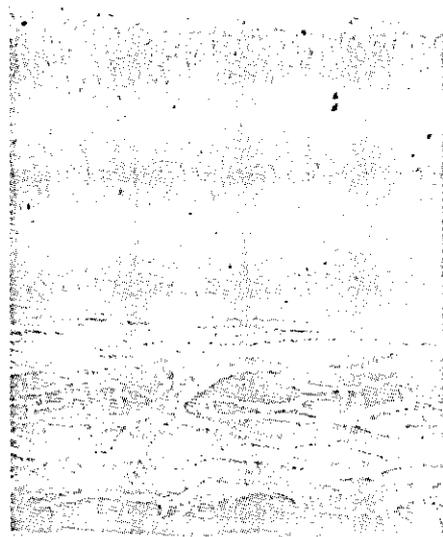
Figure 1 The porcine and human stomachs (hematoxylin-eosin stains, original magnification × 20). The wall thickness varies among different parts of the organ in the porcine stomach; the upper third of the porcine stomach is similar to the human stomach.
a The lower third of the porcine stomach.

c The upper third of the porcine stomach.

a

c

b The middle third of the porcine stomach.
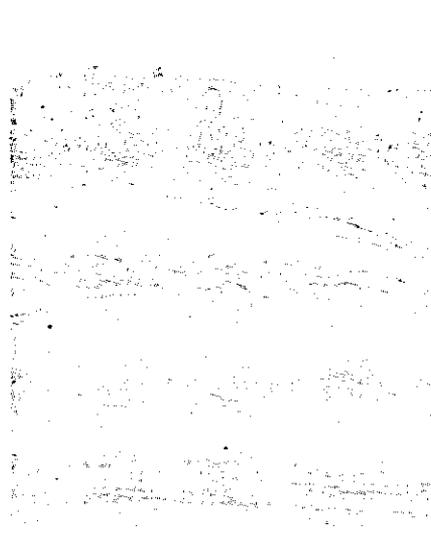
d The human stomach.

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b

d

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-

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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).
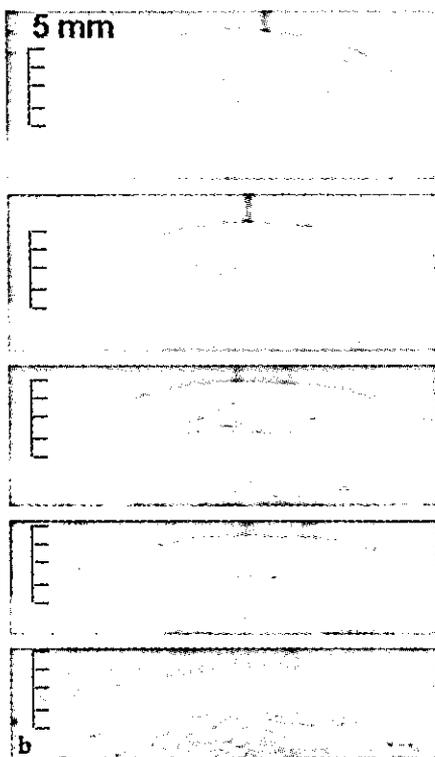
sure that the position of the specimen and the camera relative to each other remained constant and that the pictures were taken from absolutely the same angle, the specimens were set at the same distance between the edge of the fixed cork board and the fixed camera (CAMEDIA C-200 Ultra Zoom, Olympus, 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

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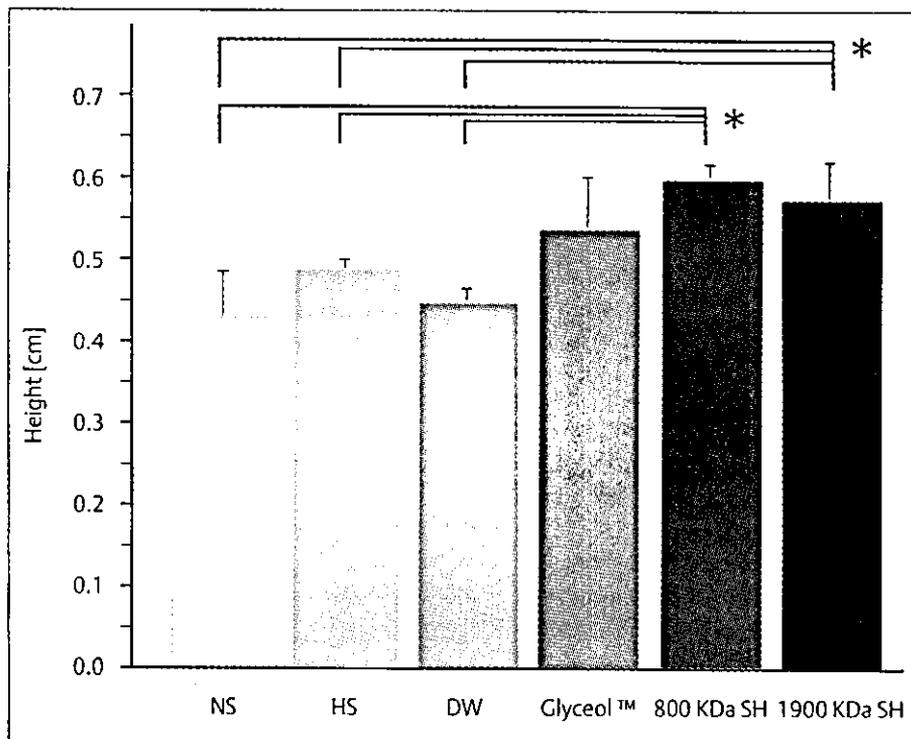
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Figure 3 The initial mucosal elevation after submucosal injection of various solutions. Both sodium hyaluronate solutions produce significantly greater elevation than normal saline (NS), hypertonic saline (HS), or 20% dextrose water (DW) (*$P<0.05$). 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.
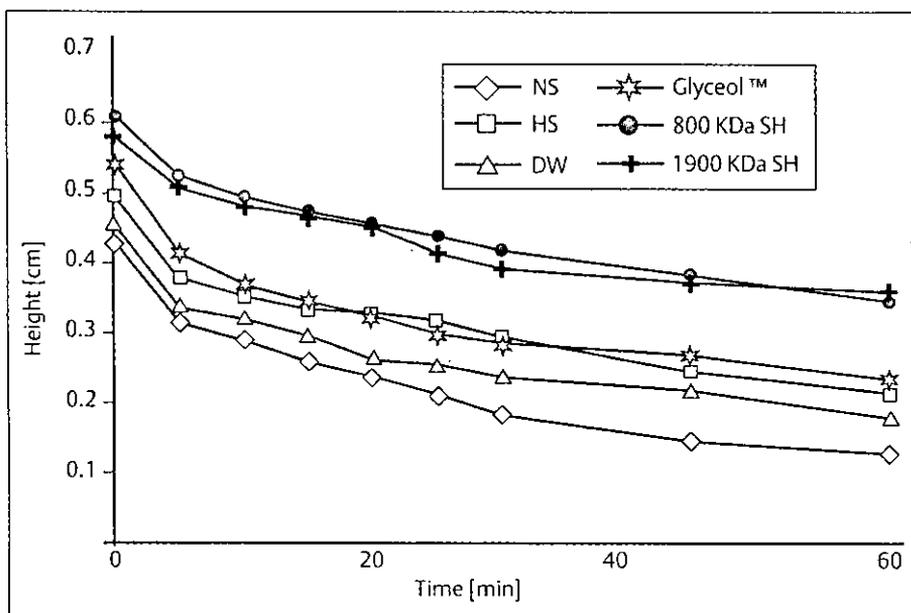

Figure 4 Chronological changes in the mucosal elevation after submucosal injection of various solutions. Both sodium hyaluronate solutions maintained a greater degree of mucosal elevation at all times than the other solutions. Hypertonic solutions maintained an intermediate degree of mucosal elevation 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.

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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

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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.

Acknowledgment

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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.

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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 osteoar-

thritis, 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).

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

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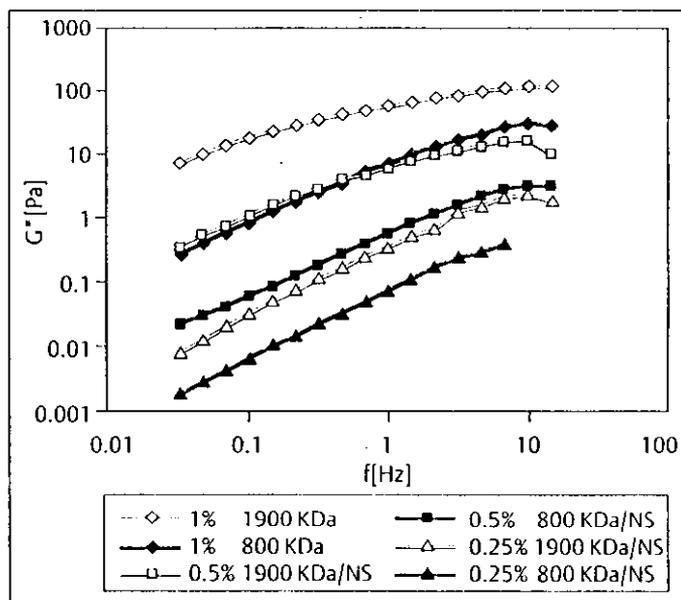
Figure 1 The viscoelasticity of sodium hyaluronate solutions mixed with normal saline. Comparison between two sodium hyaluronate (SH) preparations (800 kDa and 1900 kDa) showed that the viscoelasticity of the 800 kDa SH solutions was similar to that of 1900 kDa SH solutions at half the concentration.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

Legend for Figure 1:
- ◇ 1% 1900 KDa
- ◆ 1% 800 KDa
- □ 0.5% 1900 KDa/NS
- ■ 0.5% 800 KDa/NS
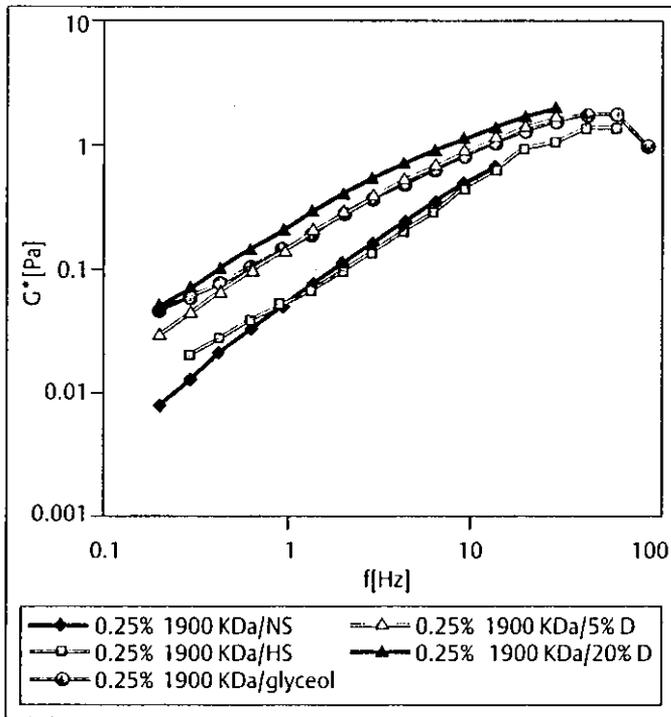- △ 0.25% 1900 KDa/NS
- ▲ 0.25% 800 KDa/NS

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 so-

lutions, 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.
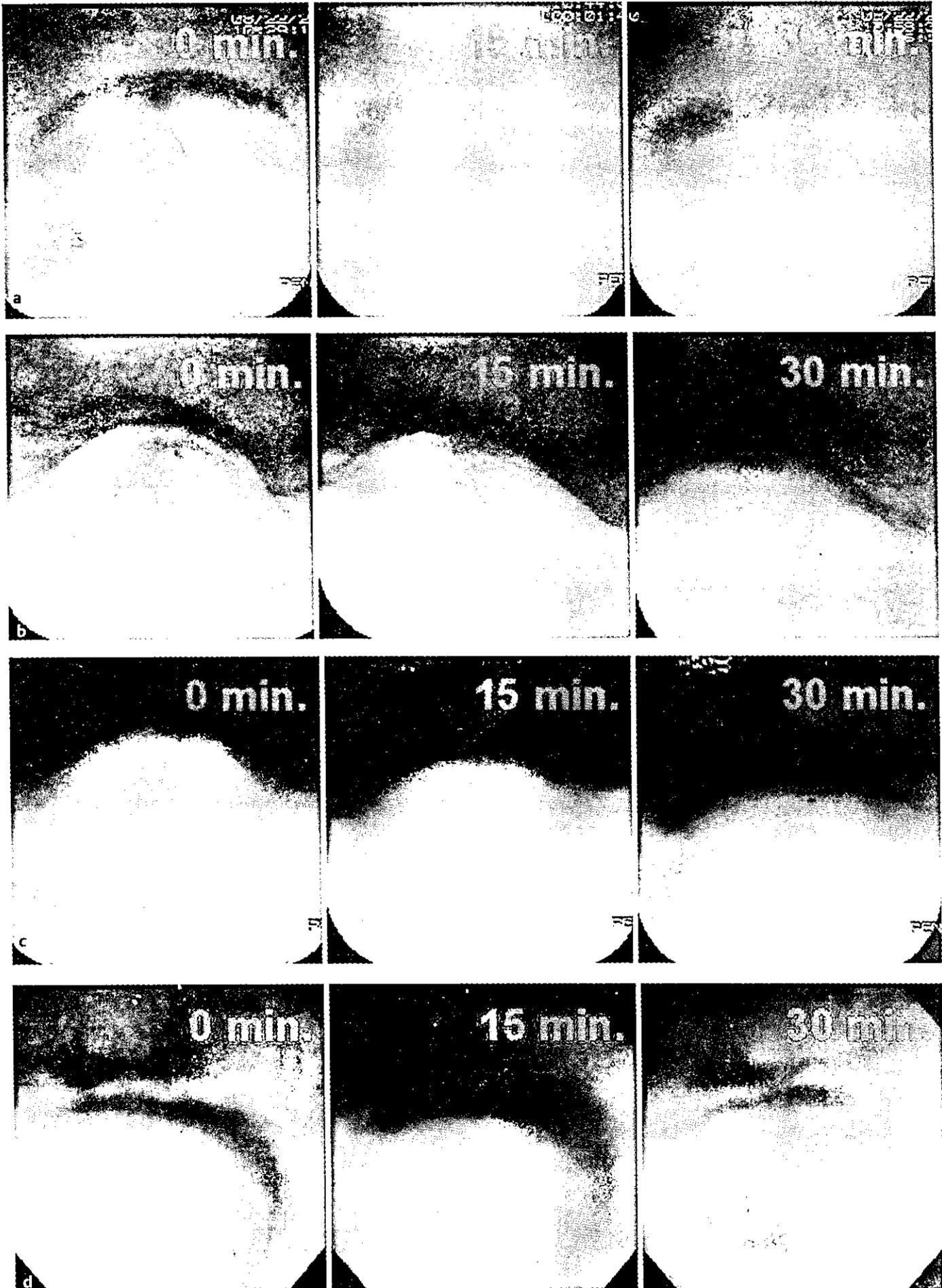
Discussion

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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