We and other researchers have shown that epithelial–mesenchymal interactions play an essential role in the control of gastrointestinal epithelial growth and differentiation not only in fetal stages, but also in adults. In the present study, we present evidence that ET-3 is an important mesenchymal factor that controls colonic epithelial growth in normal development. Then ET-3 may also play a role in regulating colonic epithelial growth in various diseases including tumors. ET-1 and its receptor have been reported to play a role in colon cancer progression (Egidy *et al.* 2000). It remains to be examined whether the function of ET-3 and its receptor are altered in colon tumors.

Acknowledgement

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ORIGINAL ARTICLE

Tissue damage of different submucosal injection solutions for EMR (CME)

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Background: When choosing submucosal injection solutions for EMR, tissue damage should be considered, as well as the lesion-lifting ability. The objective of the study was to find out the potential tissue damage of submucosal injection solutions.

Methods: The submucosal injection solutions examined were the following: normal saline solution (NS), 3.75% NaCl, 20% and 50% dextrose water (DW), a glycerin solution (Glyceol; 10% glycerin with 0.9% NaCl plus 5% fructose), and two hyaluronic acid (HA) solutions (0.25% 1900 kDa/NS solution and 0.125% 1900 kDa/ Glyceol solution). Furthermore, DW with different concentrations (5%, 10%, 15%, 30%, 40%) also was examined to find out the tolerable concentration without tissue damage. A total of 2 mL of each solution per stomach were injected by endoscopy into the submucosal layer at the separate sites of 4 living minipigs. Two minipigs were euthanized after 30 minutes of endoscopic observations, and the others were euthanized after additional endoscopic observations a week after injection.

Results: There was no apparent tissue damage in NS, 5% and 10% DW, Glyceol, or two solutions of HA, whereas, hypertonic solutions, except Glyceol and 10% DW, have more or less potency of tissue damage. In 3.75% NaCl and DW with concentrations of $\geq$20%, considerable tissue damage was observed, which might affect resected EMR specimens and ulcer healing.

Conclusions: Use of hypertonic solutions except Glyceol is not recommended with respect to tissue damage. A combination of HA and Glyceol is the most favorable submucosal injection solution, considering tissue damage and lesion-lifting ability. (Gastrointest Endosc 2005;62:933-42.)

For the purpose of preventing perforation, fluid injection into the submucosa is commonly performed during EMR to create a fluid cushion between the lesion and the muscle layer. Although the duration of lesion lifting is crucial to achieve successful results of EMR, knowledge as to tissue damage, depending on the submucosal injection solutions, should also be important. If the submucosal injection solution we choose has a high enough property of tissue damage to destroy the resected specimens, it may be difficult to make a precise histologic diagnosis of the targeted lesion. Furthermore, tissue damage of the muscle layer may result in delayed bleeding or perforation, especially in the case of the thin gut wall, such as the large and small intestines. However, there is little knowledge about the potential tissue damage of the submucosal injection solutions we widely use in clinical practice. This study compared the tissue damage of these solutions and dextrose solutions with different concentrations, by using a living minipig stomach to find out the appropriate use of submucosal injection solutions.

See CME section; p. 949.

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MATERIAL AND METHODS

Endoscopy was carried out with standard endoscopes (GIF XQ230; Olympus Optical Co, Ltd, Tokyo, Japan; and EG2931; Pentax Corp, Tokyo, Japan) in 4 minipigs (*Sus scrofa*; Miniature Swine; Chugai Research Institute for Medical Science, Inc, Nagano, Japan) that were fasted

overnight and then placed in the left lateral decubitus position after tracheal intubation and induction with general anesthesia. A disposable 23-gauge catheter injection needle (NM-200L-0423; Olympus) was used to inject 2 mL of each solution per stomach into the submucosal layer at separate sites. After injection, endoclipping was made near the injection sites to identify the locations.

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 solutions examined were the following: 0.9% NaCl (normal saline solution [NS])[1]; 3.75% NaCl (hypertonic saline solution [HS])[2]; 20% dextrose water (DW)[3]; 50% DW[4]; 10% glycerin with 0.9% NaCl plus 5% fructose (Glyceol; Chugai Pharmaceutical Co, Tokyo, Japan),[5] which is widely used in Japan for the treatment of intracranial hypertension as a formulation for intravenous drip infusion[6]; two solutions of hyaluronic acid (HA) (0.25% 1900 KDa HA solution made by a 1% 1900 KDa preparation [Suvenyl; Chugai Pharmaceutical] and NS) and 0.125% 1900 KDa HA solution made with Suvenyl and Glyceol, which have the similar ability of lesion lifting[7]; and DW with five different concentrations (5%, 10%, 15%, 30%, and 40%), which also were tested to find the tolerable concentration without tissue damage because it was thought that hypertonic solutions might have the possibility of tissue damage to some extent.

The endoscope was kept in the stomach to allow observation of the injection sites for up to 30 minutes, and two minipigs were euthanized after the observations were completed to investigate the immediate tissue damage. The other two minipigs recovered from the general anesthesia and then follow-up endoscopies were carried out a day and a week after injection. When the endoscopic observations of a week after injection were completed, the remaining two minipigs were euthanized to investigate the delayed tissue damage.

The retrieved stomachs were stretched flat on a cork board with pins and then were fixed with formalin; then the stomach was cut at the separate injection sites and embedded in paraffin. Histologic sections were made from each block and were stained with H&E, and the effect of the injections on the tissue was examined microscopically.

RESULTS

Endoscopic observation

Endoscopic observations are presented in Figure 1A to H. There was little difference among the minipigs in mucosal changes caused by the same solutions. In NS, 5% and 10% DW, Glyceol, and two HA solutions, there were no apparent mucosal changes seen by endoscopy. Hypertonic solutions, except Glyceol and 10% DW, have greater or lesser degrees of tissue damage. In 15% DW, mucosal whiteness with marginal redness at the small areas of the injection sites, not all the seeping area of the solutions, was visualized within 30 minutes. A day after the injection, the mucosal redness at the injection sites was also recognized; but, a week after injection, it was difficult to point out the injection sites by endoscopy. In HS and 20% DW, the mucosal whiteness with marginal redness at all the seeping areas of the solutions was visualized within 20 minutes. A day after injection, the mucosal redness at the all seeping areas, (in the case of HS, the central erosion was visualized) also was recognized, and, a week after injection, mucosal erosion was formed. In 30%, 40%, and 50% DW, the mucosal whiteness with marginal redness at all the seeping areas of the solutions was visualized within 15 minutes. Shallow ulceration was formed a day after injection and was persistent a week after injection.

Capsule Summary

What is already known on this topic

- Previous studies of submucosal injection solutions for EMR have evaluated only the ability to create a submucosal fluid cushion and not the potential for tissue damage.
- Recommended submucosal solutions are the following: hyaluronic acid, 50% dextrose water, and Glyceol (10% glycerin with 0.9% NaCl plus 5% fructose).

What this study adds to our knowledge

- In an animal study of available submucosal injection solutions, a mixture of high-molecular weight hyaluronic acid and Glyceol provide optimal lesion-lifting ability with minimal tissue damage.

Histologic observation

There was little difference among minipigs in tissue damage caused by the same solutions (Figs. 2A to H and 3A to D). None of NS, 5% and 10% DW, Glyceol, or two HA solutions caused any apparent tissue damage as seen by histology. In 15% DW, slight degradation of epithelial glands with mild congestion of capillary blood vessels in the superficial mucosal layer was observed in the minipigs euthanized on the day of injection, whereas, no tissue damage was observed by histology in the minipigs euthanized a week after injection. In HS and 20% DW, acute mucosal erosion with degradation of epithelial glands and congestion of capillary blood vessels was observed in the minipigs euthanized on the day of injection, and these tissue damages were persistent as mucosal erosion with fibrosis of the submucosal layer a week after injection. In 30%, 40%, and 50% DW, not only mucosal damage but also muscle damage emerged on the day of injection, and ulceration extending to the submucosal layer formed a week after injection.

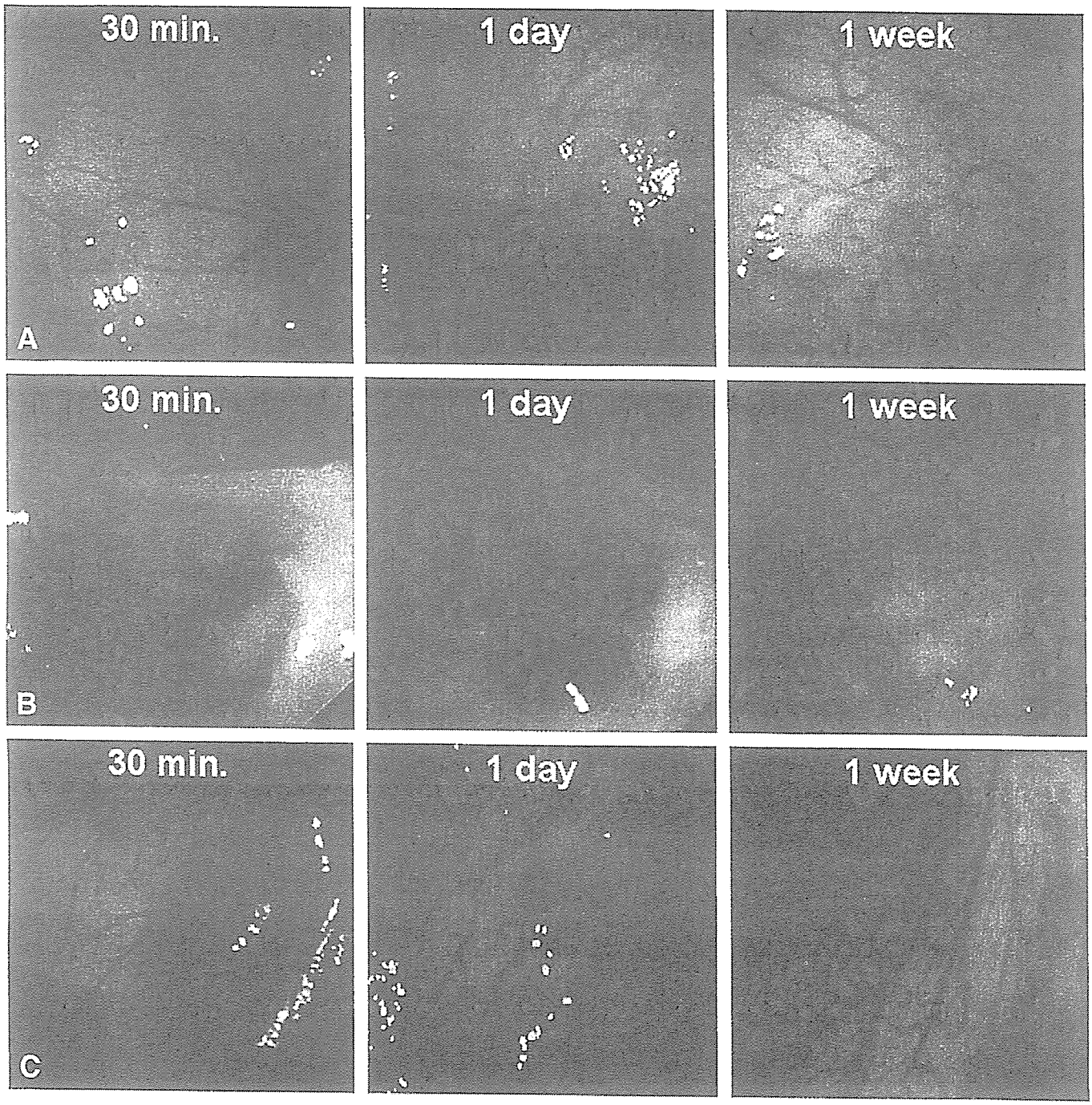

Figure 1. Endoscopic views of injection sites of various solutions. **A,** 0.9% NaCl (normal saline solution). **B,** 3.75% NaCl (hypertonic saline solution). **C,** 15% dextrose water. (*continued*)

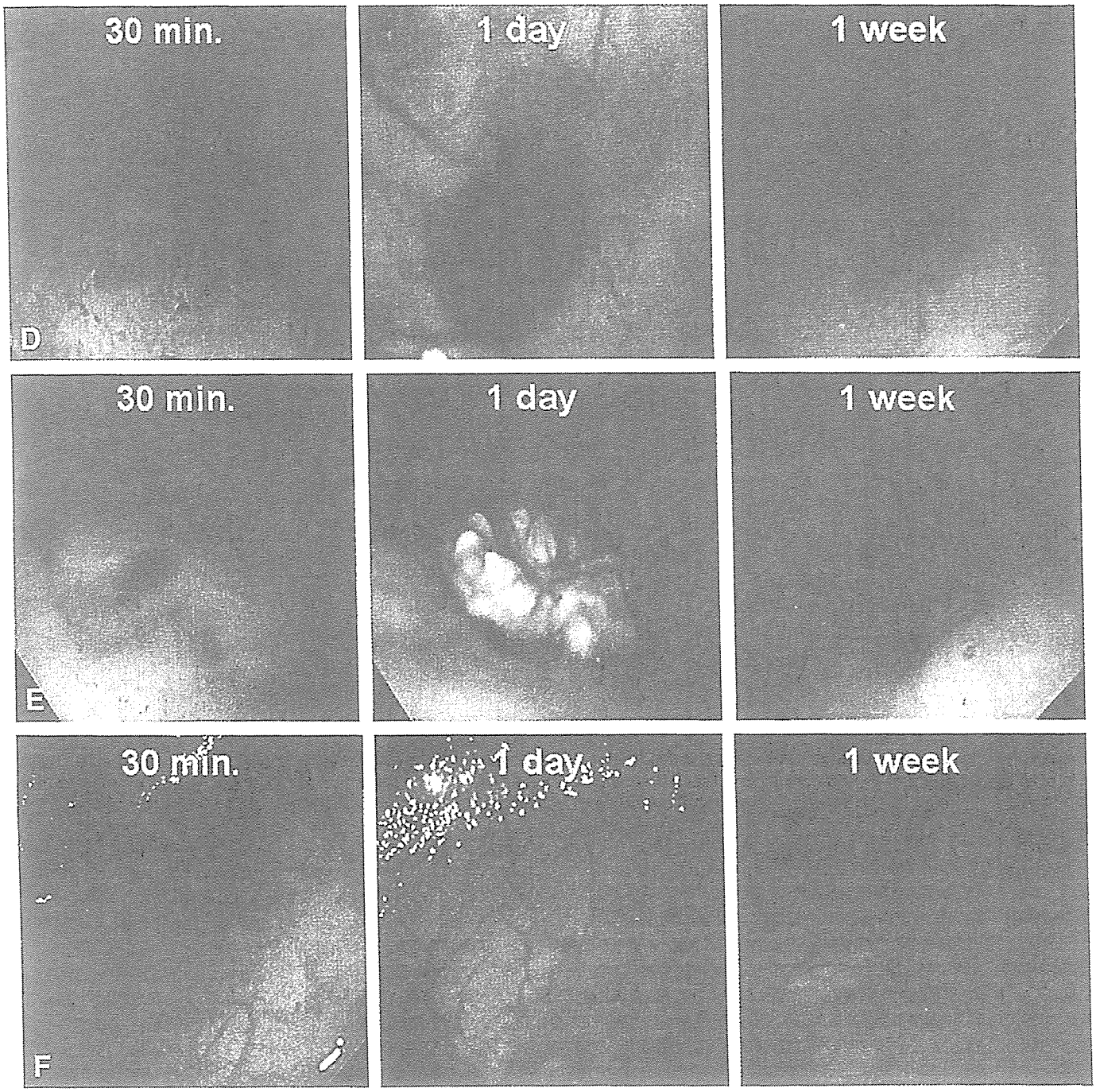

Figure 1. D, 20% dextrose water. **E,** 50% dextrose water. **F,** Glyceol (10% glycerin with 0.9% NaCl plus 5% fructose). (*continued*)

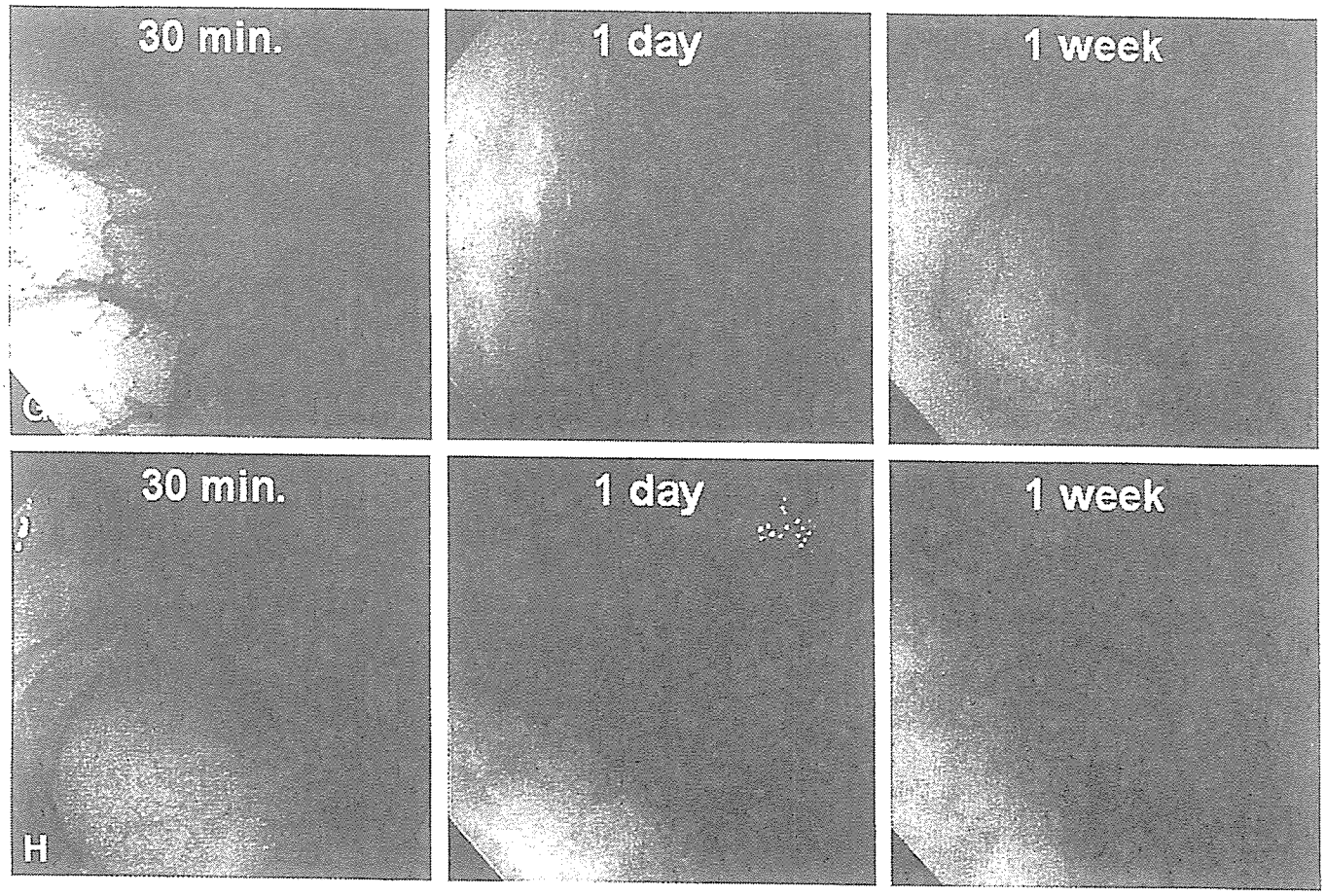

Figure 1. G, 0.25% solution of 1900 kDa hyaluronic acid with normal saline solution. **H,** 0.125% solution of 1900 kDa hyaluronic acid with Glyceol. Dextrose water with a concentration of ≥15% and hypertonic saline solution have greater or lesser degree of mucosal change. Normal saline solution, Glyceol, and two hyaluronic acid solutions have no apparent mucosal changes.

DISCUSSION

EMR has developed as a less invasive local treatment for GI tumors, which enables the preservation of the whole organ and brings about a much better quality of life for the patients with tumors.[8] In the case of the stomach, smaller intramucosal tumors without ulcer findings have been candidates for EMR until recently[9-12]; however, these days larger intramucosal tumors without ulcer findings and smaller intramucosal tumors with ulcer findings have been treated by EMR. The expansive indication of EMR has been lead by novel findings of node-negative tumors[13] and newly developed techniques in EMR,[3,14-18] called endoscopic submucosal dissection techniques. As indicated, lesions become larger or ulcerative, the frequencies of complications are increasing and relatively high complication rates have become other problems. In the use of an insulation-tipped knife for the treatment of gastric tumors, the rates of bleeding and perforation were reported as 22% and 5%, respectively.[19]

Several approaches to lessen these complications have been tried, and, among these, the most effective and simplest way to prevent complications, especially perforation, is to maintain a sufficiently thick submucosal layer for a time by endoscopic injection of fluid into the submucosa. The recent studies,[19-21] including ours, revealed that HA solutions were the best available solutions in creating the thickest fluid cushion and that hypertonic solutions tended to create higher lesion lifting than NS. These studies recommended the alternative use of hypertonic solutions in practice, because HA is much more expensive than the other solutions.

Although hypertonic fluid may be better for creating higher lesion lifting and for obtaining effective hemostasis,[22] potential tissue damage by submucosal injection may be considered, because of its high osmolality. As shown in this study, the concentration of less than 15% in DW, whose osmotic pressure is 3 times higher than the extracellular fluid, is recommended as a submucosal injection solution with respect to tolerable tissue damage, if we want to have resected specimens with good quality and avoid unexpected delayed healing of the artificial ulcers after EMR. It is speculated that Glyceol, which consists of 10% glycerin, 0.9% NaCl, and 5% fructose, has

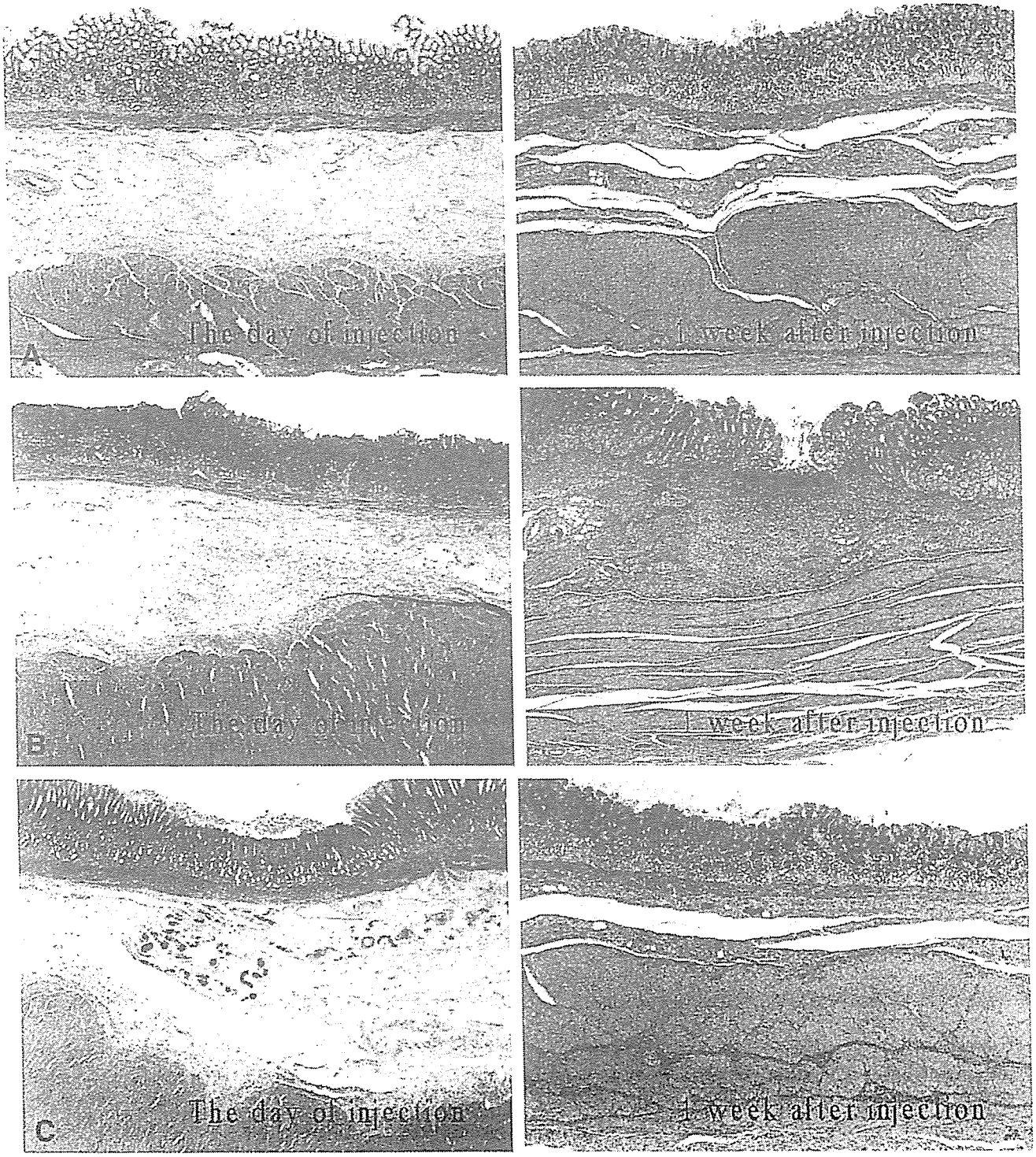

Figure 2. Histologic changes of injection sites of various solutions. **A,** 0.9% NaCl (normal saline solution) (H&E, orig. mag. ×40). **B,** 3.75% NaCl (hypertonic saline solution) (H&E, orig. mag. ×40). **C,** 15% dextrose water (H&E, orig. mag. ×40). (*continued*)

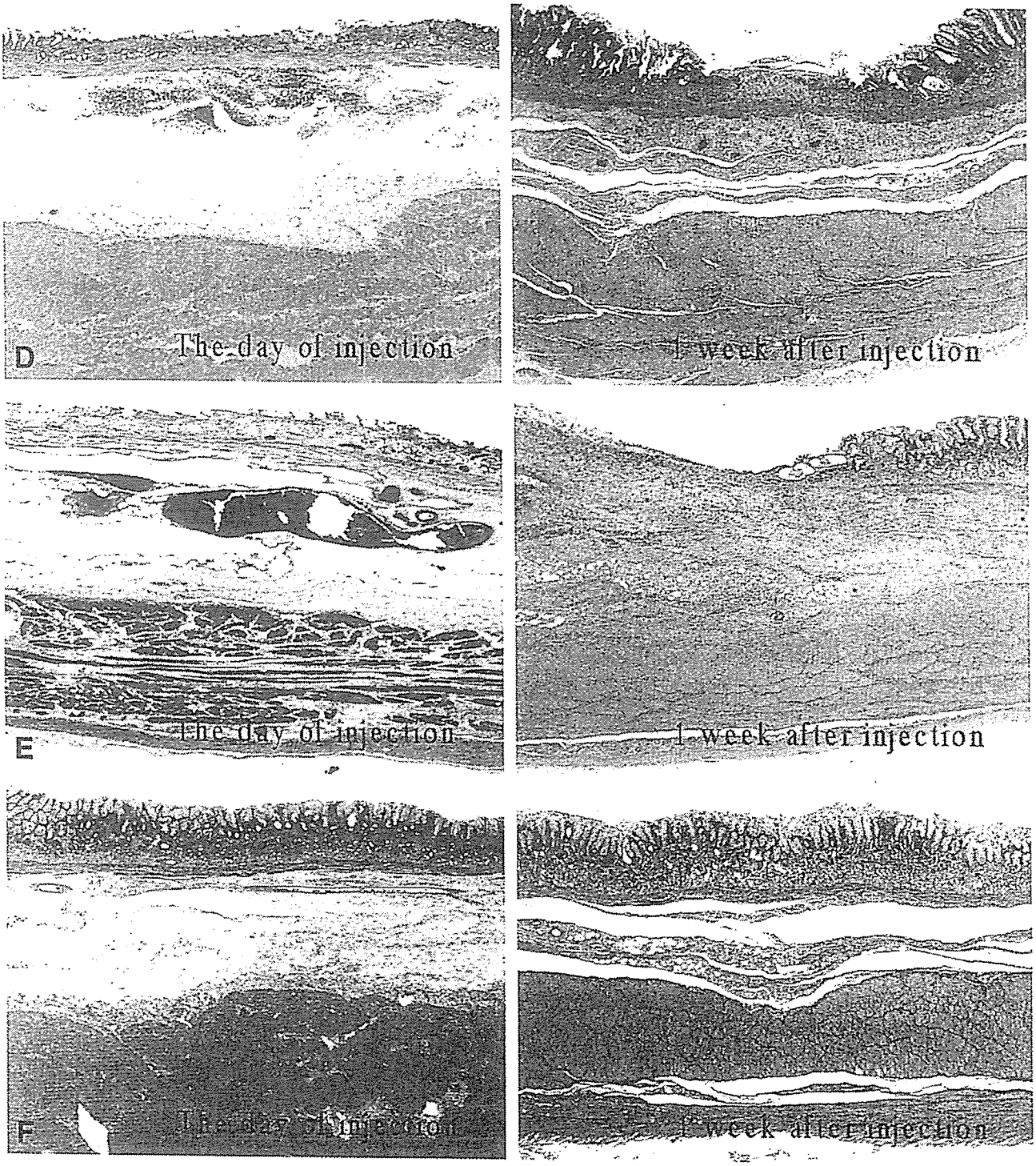

Figure 2. D, 20% dextrose water (H&E, orig. mag. ×40). **E,** 50% dextrose water (H&E, orig. mag. ×40). **F,** Glyceol (10% glycerin with 0.9% NaCl plus 5% fructose) (H&E, orig. mag. ×40). (*continued*)

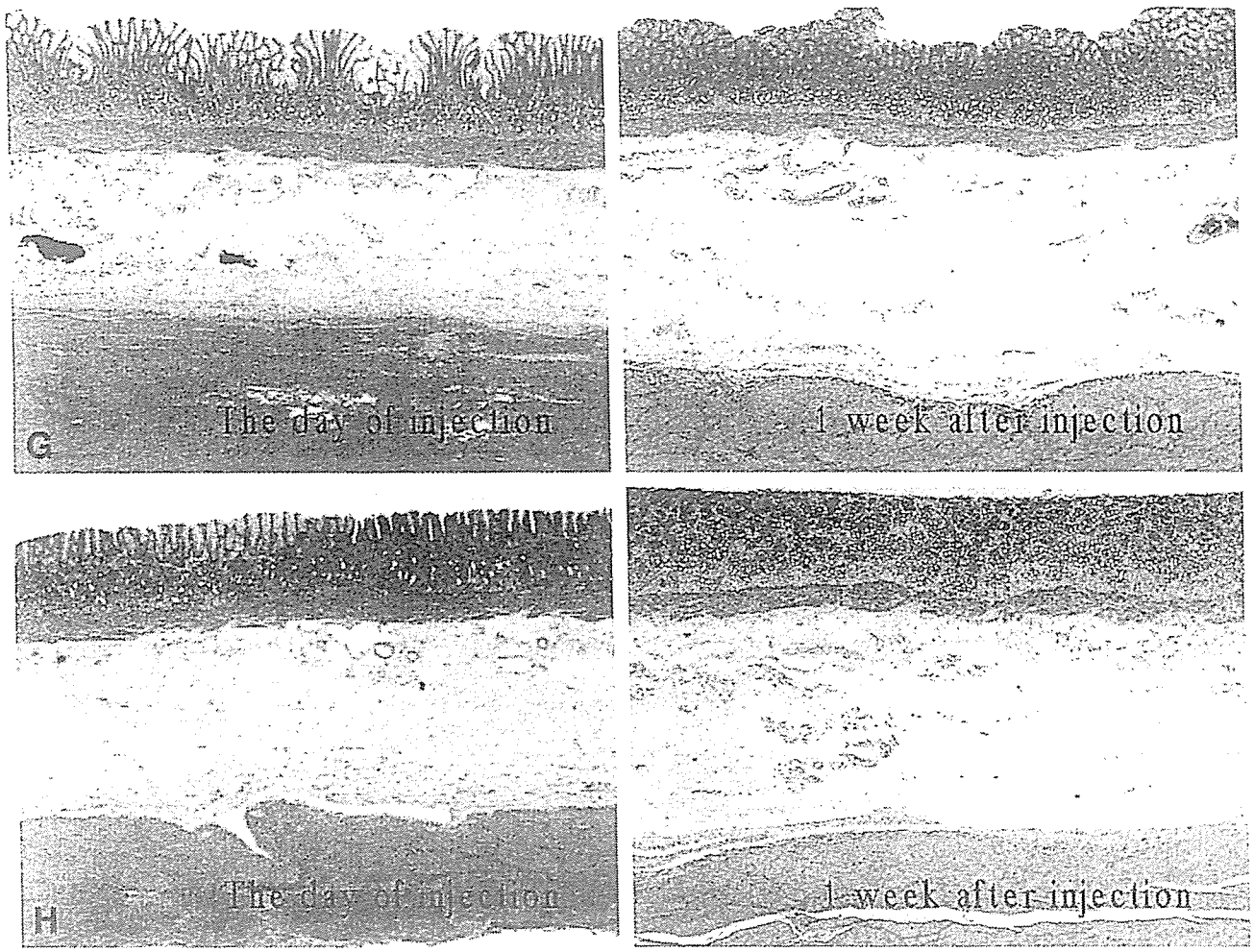

Figure 2. G, 0.25% solution of 1900 kDa hyaluronic acid with normal saline solution (H&E, orig. mag. ×40). **H,** 0.125% solution of 1900 kDa hyaluronic acid with Glyceol (H&E, orig. mag. ×40). Dextrose water with concentration of ≥15% and hypertonic saline solution have greater or lesser degree of mucosal damage, and 50% dextrose water causes tissue damage not only in the mucosal layer but also in the proper muscle layer, which is revealed by histologic assessment of the stomachs of minipigs euthanized immediately after endoscopic observations of the day of injections. Those mucosal damages do not reverse a week after injection except 15% dextrose water and mucosal erosion or ulceration is formed. Normal saline solution, Glyceol, and two hyaluronic acid solutions have no apparent mucosal changes.

no apparent tissue damage because glycerin can pass freely through the cell membrane, because the osmotic pressure difference between the inside and the outside of the cell membrane is only generated by an additional use of 5% fructose, which causes no cell destruction. On the contrary, because osmotic pressure of Glyceol is approximately 7 times higher than the extracellular fluid, Glyceol has a possibility to produce sufficient submucosal cushion.

Among available solutions, HA solutions may be the best as a submucosal injection solution with regard to tissue damage, as well as to its lesion-lifting ability. HA is a thick substance, with high viscoelasticity widely found in connective tissues. Its current approved indications in clinical practice are intra-articular injections for osteoarthritis and use in eye surgery in many countries, including Japan, Europe, and the United States. It is not antigenic or toxic to humans,[23-25] and only minor adverse effects are reported in clinical use.[26] However, the crucial disadvantage of HA solutions may be their high costs. A previous study[7] of ours revealed that a combination of high-molecular-weight HA and Glyceol is an ideal submucosal injection solution with an acceptable cost, considering the facts of increasing viscoelasticity of HA hypertonicity to create sufficient lesion-lifting effect without tissue damage.[7] If the cost of the HA solution is also unacceptable, Glyceol alone is more readily available and may be a practical alternative to HA solutions. The disadvantage of a slipping submucosal cushion by the solutions, with less lesion-lifting ability could be

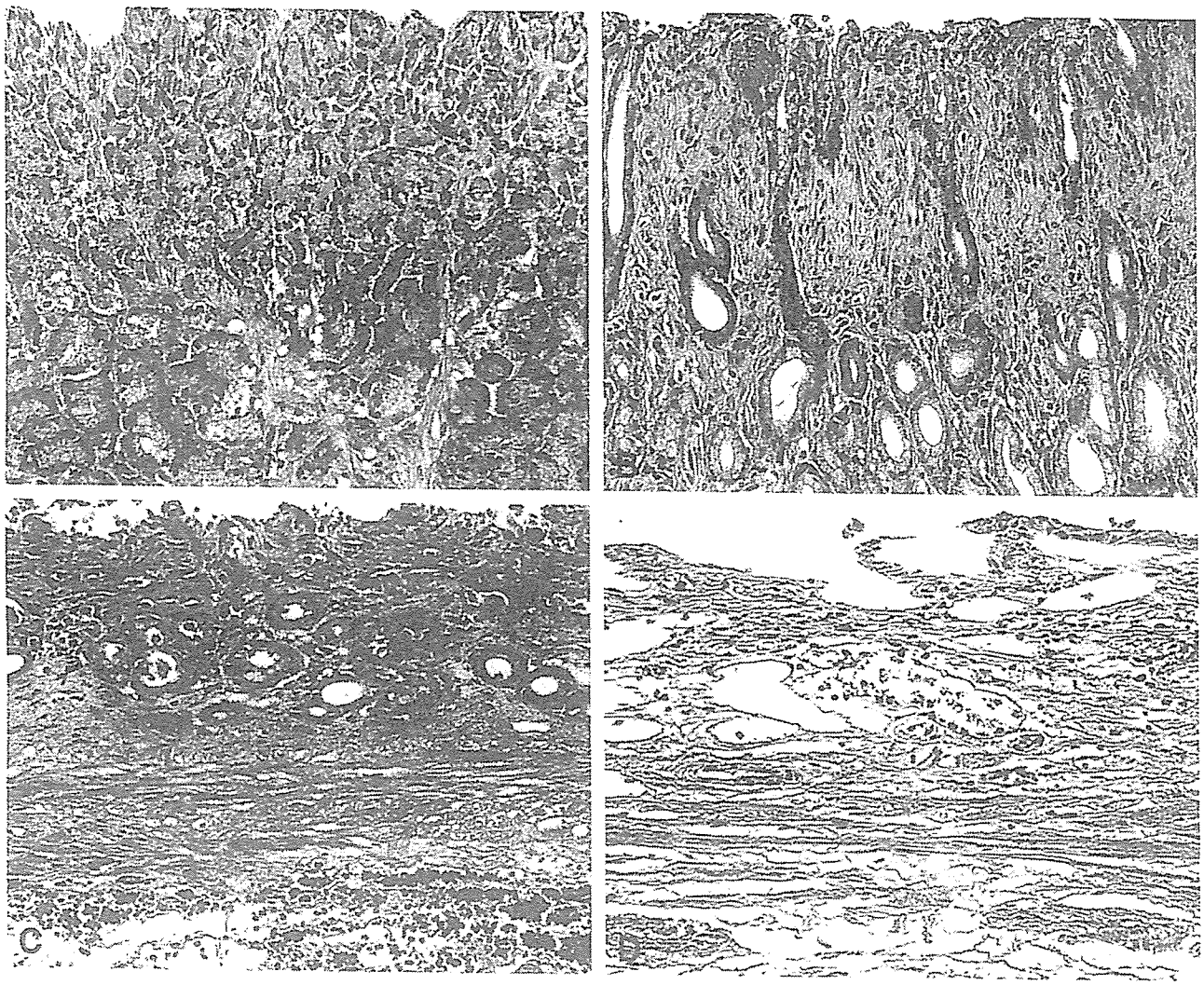

Figure 3. Histologic mucosal damages of hypertonic solutions. **A,** 3.75% NaCl (hypertonic saline solution) (H&E, orig. mag. ×200). **B,** 15% dextrose water (H&E, orig. mag. ×200). **C,** 20% dextrose water (H&E, orig. mag. ×200). **D,** 50% dextrose water (H&E, orig. mag. ×200). Partial degradation of epithelial glands and congestion of capillary blood vessels are observed in hypertonic saline solution, and 15% and 20% dextrose water. Furthermore, 20% dextrose water causes erosion of the superficial mucosal layer. In 50% dextrose water, complete degradation of epithelial cells and destruction of the mucosal structure are observed.

balanced by repeated injection of the solutions during the procedure. This also would be an alternative for the use of HA solutions or Glyceol, but there is no data available that indicates that repeated injection of a solution with less lesion-lifting ability, such as NS, achieves similar outcomes with a single injection of a solution with more lesion-lifting ability, such as HA solutions or Glyceol.

Finally, we emphasize from this study, that a submucosal injection solution should be chosen considering tissue damage as well as lesion-lifting ability to perform successful EMR with good quality. On the basis of experimental studies, including this study, a prospective clinical trial may be planned in the future, and it is necessary to find out the advantage of HA solutions or Glyceol over NS in the clinical practice.

DISCLOSURE

Koji Kashimura in a member of the Product Research Department, Kamakura Research Laboratories, Chugai Pharmaceutical Co, Ltd, Kanagawa, Japan; Toyokazu Matsuura is a member of the Chugai Research Institute for Medical Science, Inc, Nagano, Japan.

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PROGRESSION OF CHRONIC ATROPHIC GASTRITIS ASSOCIATED WITH *HELICOBACTER PYLORI* INFECTION INCREASES RISK OF GASTRIC CANCER

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We conducted a longitudinal cohort study to determine the association of *Helicobacter pylori* infection and the progression of chronic atrophic gastritis (CAG) with gastric cancer. A cohort of 4,655 healthy asymptomatic subjects was followed for a mean period of 7.7 years. *H. pylori* infection was established by serum specific antibodies and the presence of CAG was confirmed by serum pepsinogen. During the follow-up period, 45 gastric cancer cases were detected (incidence rate, 126/100,000 person-years). A univariate analysis after adjustment for age showed that both *H. pylori* and CAG were significantly associated with gastric cancer. To clarify the interaction between *H. pylori* and CAG, an analysis stratified by *H. pylori*- and CAG-status was performed. No cancer developed in the *H. pylori*(−)/CAG(−) group during the study period. This supports the theory that it is quite rare for any type of gastric cancer to develop in an *H. pylori*-free healthy stomach. With the progression of *H. pylori*-induced gastritis, the risk of gastric cancer increased in a stepwise fashion from CAG-free gastritis [*H. pylori*(+)/CAG(−) group] (HR=7.13, 95%CI=0.95-53.33) to CAG [*H. pylori*(+)/CAG(+) group] (HR=14.85, 95%CI=1.96–107.7) and finally to severe CAG with extensive intestinal metaplasia [*H. pylori*(−)/CAG(+) group] (HR=61.85, 95%CI=5.6–682.64) in which loss of *H. pylori* from the stomach is observed. Therefore, it is probable that *H. pylori* alone is not directly associated with stomach carcinogenesis. Instead, *H. pylori* appears to influence stomach carcinogenesis through the development of CAG. The observed positive correlation between the extent of *H. pylori*-induced gastritis and the development of cancer was strong, especially for the intestinal type. These results are compelling evidence that severe gastritis with extensive intestinal metaplasia is a major risk factor for gastric cancer, and they confirm the previously described model of stomach carcinogenesis: the gastritis-metaplasia-carcinoma sequence.

Key words: *atrophic gastritis; gastric cancer;* Helicobacter pylori*; cohort study; pepsinogen*

Despite a worldwide decline in incidence, gastric cancer remains one of the leading causes of cancer-related death in Japan.[1–4] There is a marked geographic variability in the gastric cancer incidence rate; the cancer is most common in China and Japan, and one of the lowest rates is in the United States.[1–4] Many epidemiologic studies have shown that the risk of gastric cancer is strongly associated with environmental factors, such as salt, nitrates and low intake of fresh fruits and vegetables.[1,4–8] Recent studies have indicated that *Helicobacter pylori* infection is also a major risk factor for the development of gastric cancer.[9–18] The prevalence of *H. pylori* infection is markedly higher in Japan than in other industrialized countries, although the reasons are not fully understood.[19–21] The observed geographic variability in gastric cancer appears to be explained by a synergistic interaction between *H. pylori* infection and other environmental factors.

The *H. pylori* bacterium colonizes the stomach mucosa and triggers a series of inflammatory reactions. It is considered an important cause of chronic atrophic gastritis (CAG),[19–23] as shown in rodent models.[24–26] CAG is considered the first step of a sequence of mucosal changes in the stomach leading to cancer. The current model for stomach carcinogenesis begins with gastritis, proceeds to CAG, then to intestinal metaplasia, dysplasia and, finally, carcinoma.[1,27] This hypothesis is supported by a considerable number of clinicopathological and epidemiological studies in countries with a high incidence of gastric cancer. However, longitudinal cohort studies that report an association of CAG with gastric cancer and a relation between the progression of CAG and the development of gastric cancer are limited.[28–30] In addition, the role of *H. pylori* infection in the above-mentioned process of stomach carcinogenesis remains unclear. To investigate these problems relating to gastric cancer development, we established a cohort of male factory workers that we followed prospectively for 8 years.

CAG in a high-risk population, such as Japanese subjects, usually begins at the gastric antrum and extends proximally towards the cardia.[31–33] As a result, gastric secretory function diminishes as the area of functional fundic gland mucosa gets smaller.[34] CAG is a histopathological diagnosis. It is difficult, however, to accurately quantify the extent of CAG based on a few endoscopic biopsy samples because CAG is usually a multifocal process.[35] Our previous study showed that the reduction in the area of the fundic gland mucosa with the progression of CAG was well correlated with the stepwise reduction in the serum pepsinogen (PG) level.[34] Thus, the serum PG level is considered a reliable marker for the extent of CAG. Since the measurement of serum PG is simple to obtain and the study subjects experience no discomfort, we used the serum test to evaluate the extent of CAG in our cohort. Along with serum PG levels, we analyzed anti-*H. pylori* IgG antibodies for the evaluation of *H. pylori* infection. Using the 2 serologic markers, we determined the incidence of gastric cancer in the cohort and evaluated the risk for gastric cancer associated with *H. pylori* infection and subsequent CAG progression.

SUBJECTS AND METHODS

Study population

Subjects were 5,706 male employees, 40 to 59 years old, who underwent an annual multiphasic health checkup in a workplace in Wakayama City, Japan. Between April 1994 and March 1995, fasting blood samples were collected as routine laboratory tests for

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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). Differences 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.

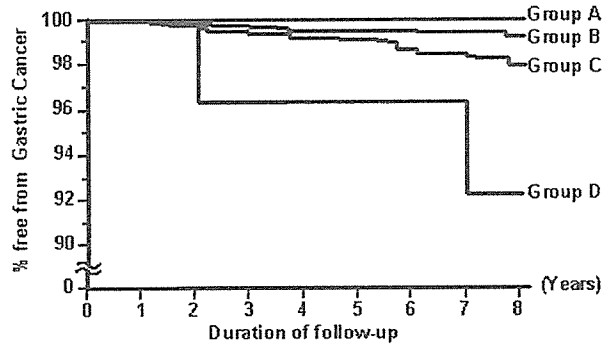

FIGURE 1 – Kaplan-Meier analysis in relation to the progression of chronic atrophic gastritis (CAG). Subjects were classified into 1 of 4 groups (A to D) based on the results of the 2 serologic tests, anti-*H. pylori* titer and serum pepsinogen level. Group A [*H. pylori*(−)/CAG(−)], infection free subjects; 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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An Unusual Case of Polypoid Angiodysplasia

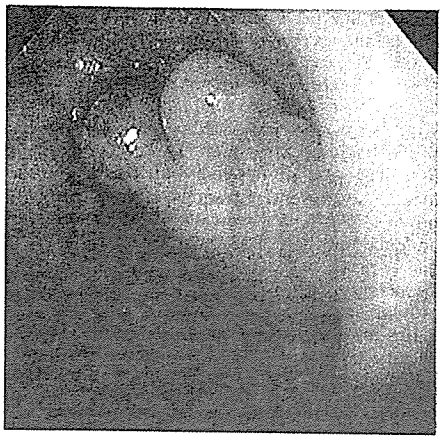

Figure 1 A healthy 59-year-old man underwent colonoscopy because of a positive fecal blood test. A yellowish-white lobulated polyp, 15-mm in size with a "baby's hand"-like morphology, was seen. Endoscopic polypectomy for suspected leiomyoma was performed without complication.

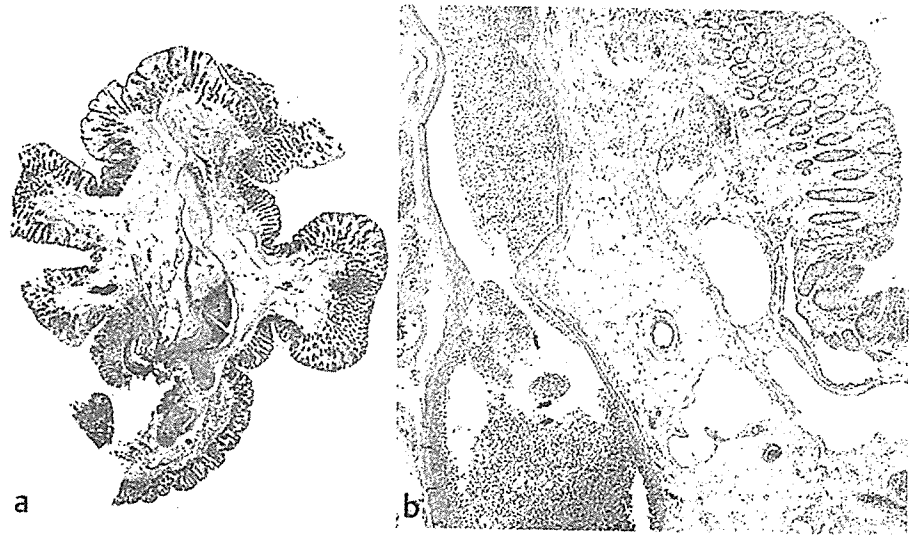

Figure 2 The edematous stroma was filled with enlarged serpiginous veins and arterioles, leading to a diagnosis of angiodysplasia. Angiodysplasia is one of the major causes of lower gastrointestinal bleeding, often encountered during emergency colonoscopy. The typical endoscopic appearance is often reported 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.

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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.