

RESEARCH COMMUNICATION

Endoscopic Observation of N-Methyl-N'-Nitro-N-Nitrosoguanidine-Induced Gastric Carcinogenesis in Rat Using A Newly-Developed Flexible Endoscope

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Abstract

Endoscopy can be used for sequential observation of gastric carcinogenesis in animal models. In the present study, we applied endoscopic examination and biopsy technique on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced stomach cancer in rats using a newly-developed flexible 2.5 mm endoscope. A total of 36 rats were divided into MNNG-treated and non-treated groups, observed on gastric endoscopy every 5 weeks, and sacrificed at week 10, 25, 35, and 50. The sequential growth process of MNNG-induced gastric tumor was clearly found by the endoscopic examination. Endoscopic appearances including incidence and size of tumor were well consistent with histological findings. In addition, biopsy specimens could be extracted from gastric mucosa in living rats using a biopsy forceps. These results indicate that the endoscopic technique can be a useful tool for investigating gastric carcinogenesis by sequential observation and collection of biopsy specimens.

Key Words: Endoscopy - gastric cancer - N-methyl-N'-nitro-N-nitrosoguanidine model - rat

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Introduction

Stomach cancer is the fourth most common cancer and second leading cause of cancer-related death worldwide (Parkin et al., 2005). Several rodent models have been developed for the investigation of gastric carcinogenesis (Tsukamoto et al., 2007). However, these rodent models are difficult to perform sequential observation of stomach tumor at an individual level and need a lot of animals to compare the results at varying time points. Gastric endoscopy is the most important examination for diagnosis and grading of stomach cancer and other gastric disorders in humans. Nevertheless, there has been limited endoscopic investigation of gastric carcinogenesis using rodent models, because of restrictions of size and narrow viewing field by rigid endoscope.

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats has proved to be good experimental models for human stomach cancer of the differentiated type (Sugimura and Fujimura, 1967; Saito et al., 1970). In the present study, we performed sequential observation of MNNG-induced gastric carcinogenesis in rats using a newly-developed gastric endoscopy and

biopsy forceps for rodents. We examined the endoscopic and histopathological findings and evaluated the availability of the model.

Materials and Methods

Experimental design

The experimental design is illustrated in Figure 1A. A total of 36 specific pathogen-free male, 7-week-old Sprague-Dawley rats (*Rattus norvegicus*; Clea Japan, Tokyo, Japan) were used. All animals were housed in plastic cages on hardwood-chip bedding in an air-conditioned biohazard room with a 12-h light/12-h dark cycle, and allowed free access to food and water. The rats were divided into 2 groups (Groups A and B). Animals of Group A were administered MNNG (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) at the concentration of 150 µg/ml for 25 weeks via light-shielded bottles in drinking water ad libitum. MNNG solutions were freshly prepared 3 times per week. Animals were sacrificed at weeks 10 (Group A, n=7), 25 (Group A, n=7), 35 (Group A, n=6), and 50 (Group A, n=11; Group B, n=5), and compared the endoscopic and histological findings. The

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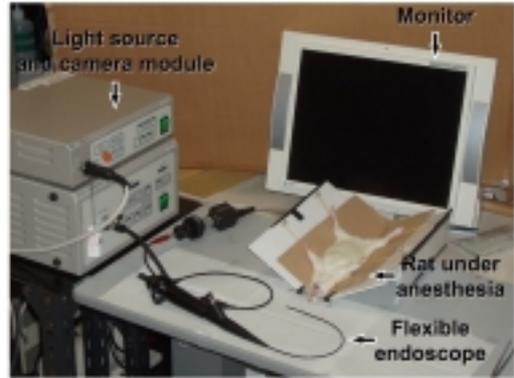
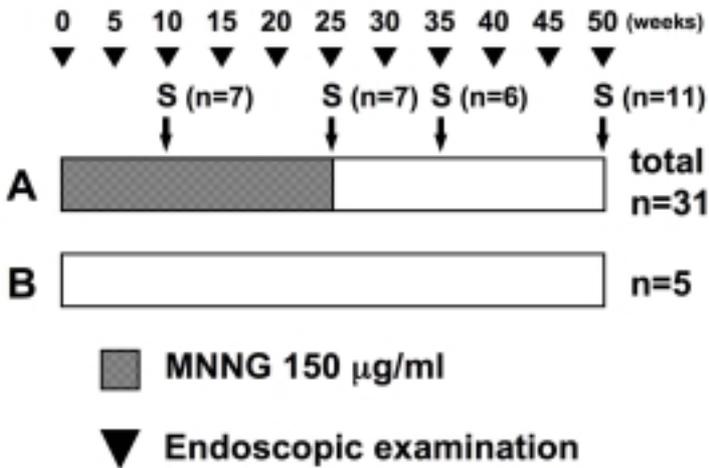


Figure 1. Experimental Details. (Left) Experimental design. Seven-week-old male rats were administered tap water or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in their drinking water for 25 weeks, and examined by gastric endoscopy every 5 weeks. (Right) Experimental set up of the gastric endoscopy

experimental designs were approved by the Animal Care Committee of the Aichi Cancer Center Research Institute, and the animals were cared for in accordance with institutional guidelines as well as the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

Endoscopic examination

In the experimental period, all animals were observed

on gastric endoscopy every 5 week. After 24 hours fasting, rats were anesthetized by inhalation of diethyl ether and intraperitoneal injection of 120 mg/kg 2, 2, 2-tribromoethanol (Sigma-Aldrich, St Louis, MO, USA). To prevent the airway obstruction, tracheal intubation was performed with a special laryngoscope. A flexible endoscope with 2.5 mm outer diameter, 180/120° up/down range of motion and a 1.2 mm working channel (Machida Endoscope Co. Ltd., Tokyo, Japan) was used for

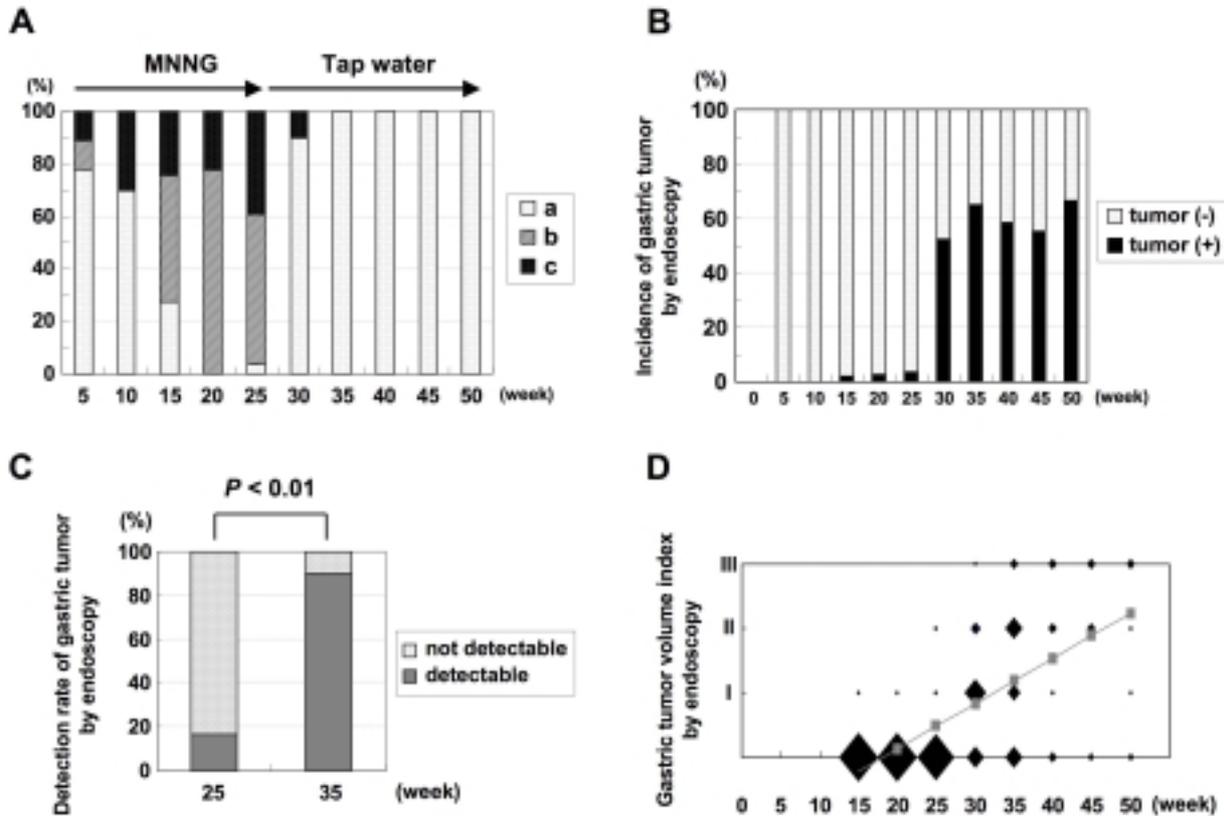


Figure 2. Lesion Details. (A) Relationship between time course and mucosal lesions of glandular stomach. Endoscopic mucosal lesions were classified into 3 types; (a) No lesions, (b) mild bleeding, (c) redness or erosion. (B) Relationship between incidence of gastric tumor by endoscopy and time course. (C) Proportion of detectable tumor by endoscopy. P value was calculated by the Fisher's exact test. (D) Regression analysis between time course and tumor size by endoscopic examination. Endoscopic tumor size was classified into 3 types; (I) < 3 mm, (II) 3-6 mm, (III) > 6 mm. Size of rhombus reflects the number of individuals. Correlation coefficient R = 0.69.

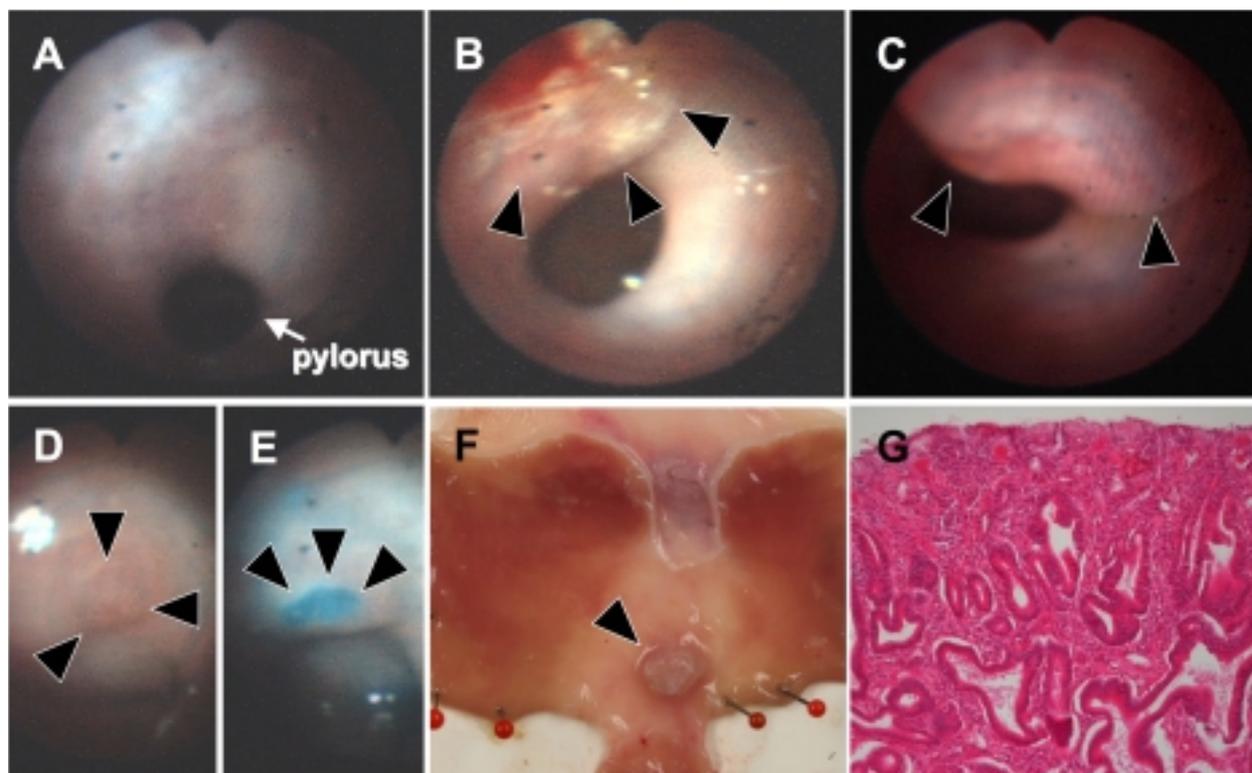


Figure 3. (A-E) Endoscopic images of rat glandular stomach. (A-C) Sequential observation of gastric tumor in an identical rat; (A) no tumor was observed at week 25, (B) early tumor with hemorrhage (arrowheads) at week 35, (C) apparent gastric tumor (arrowheads) at week 50. (D and E) Chromoendoscopy with indigocarmine in glandular stomach. (D) Although the erosive lesion indicated by arrowheads was not apparent by normal endoscopy, (E) chromoendoscopy with indigocarmine allowed the easy detection. (F) Macroscopic observation of gastric tumor (arrowhead) at week 50. (G) Biopsy specimens of glandular stomach. Well-differentiated adenocarcinoma at week 50 (H&E staining).

observation of the glandular stomach (Figure 1B). Water irrigation and air insufflation were performed through the working channel to visualize the gastric lumen when necessary. The endoscopic appearances of gastric mucosal lesions and tumor size were classified into three types respectively, (mucosal lesions) a: no lesion, b: mild bleeding, c: redness or erosion; (tumor size) I: < 3 mm, II: 3-6 mm, III: > 6 mm. Biopsy was conducted by the flexible biopsy forceps with a diameter of 0.9 mm and specimens of the gastric mucosa were obtained. Chromoendoscopy with spraying indigocarmine was also performed to help diagnosis. All endoscopic examinations were performed by the same operators (SK and AM).

Histopathological examination

For histological examination, the biopsied specimens obtained from living rats and excised stomachs from necropsy were fixed in 10% neutral-buffered formalin for 24 hours and embedded in paraffin. Sections (4- μ m thick) were prepared and stained with hematoxylin and eosin (H&E) for histological findings.

Results

In the endoscopic examination, almost all region of the glandular stomach, including the pylorus, could be visualized by our flexible endoscope. Mild to moderate mucosal lesions such as bleeding, redness, and erosion were observed in the gastric mucosa especially during

MNNG treatment. These lesions immediately disappeared after the treatment ended (Figure 2A).

At week 15, gastric tumors became macroscopically visible by endoscopic examination (Fig. 2B). Endoscopic incidence of stomach tumor in MNNG-treated rat rapidly increased after 25 weeks (4.2%), and reached 58.8 % at week 30 (Fig. 2B). However, necropsy at week 25 revealed that several flat tumors were not detectable by endoscopy at this point (Fig. 2C).

Gradually developing of tumor was observed by sequential endoscopy (Figs. 3A-C), and the tumor volume index by endoscopy was positively correlated with time course (Figure 2D). Most gastric lesions were detectable by standard endoscopy, while some of flat type lesions without apparent ulcers or polyps required the chromoendoscopy to detect (Figures 3D and E). All gastric tumors were located at the pylorus region or antrum (Figure 3F), while some tumors were found in the duodenum by the endoscopy, and the upper part of the jejunum at autopsy. Biopsy specimens were taken through the working channel of endoscopy and sections from these 0.9 mm pieces were sufficient for histological diagnosis (Figure 3G).

Discussion

In the present study, we demonstrate the use of a small animal endoscope to monitor the gastric carcinogenesis sequentially in a rat model. Experimental rat models have

been used for investigation of the pathogenesis, therapies and preventions for gastrointestinal cancer (Kitajima et al., 1992; Schwab et al., 1997). However, it has been difficult to analyse the sequential development of tumors in the same rat, because there were no means to observe the entire region of stomach mucosa in living rats. In the past, several endoscopic attempts were made to perform endoscopy in rodent models including rat esophagus (Lu et al., 2009), rat stomach (Fukawa et al., 1983; Taylor et al., 1988), and large intestine of rats or mice (Huang et al., 2002; Becker et al., 2005; Haughn et al., 2006). These endoscopies previously used for rat stomach were straight rigid type that causes the limited field of view. The newly-developed flexible endoscope with 2.5 mm in diameter used in the present study apparently overcame the difficulty. In addition, chromoendoscopy with indigocarmine helped to detect invisible lesions by standard endoscopy.

The endoscopic observations in rats were well consistent with the histological findings from biopsy and necropsy, suggesting that our biopsy forceps is useful for the sequential obtaining gastric mucosa and observation in the same living rats. Histological structures of MNNG-induced gastric cancer in rat have been known to be similar to well-differentiated gastric cancer in human (Saito et al., 1970). Recent study have found that MNNG-induced gastric tumor in rats had similar pattern of gene expression profile to human gastric cancer by oligonucleotide microarray analysis (Abe et al., 2003). We suggest that our biopsy technique may allow to analyzing the time course of gene expression in an identical tumor during carcinogenesis.

In conclusion, we demonstrated that our flexible endoscope is a useful method for sequential observation of gastric carcinogenesis in living rats without too much sacrifices. In addition, the endoscopic biopsy technique may be valuable for investigation the sequential gene expression pattern of gastric tumor in rodent models.

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