Histopathological Findings of the Lower Esophagus after Total Gastrectomy in Rat

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Summary: It is now accepted that the incidence of esophageal carcinoma is highest in the middle thoracic region. Esophageal carcinoma after gastrectomy, however, has a tendency to develop in the lower region. This study was designed to investigate the role of reflux of gastroduodenal juice in the genesis of carcinoma in the esophagus. We found a possible correlation between the development of esophageal carcinoma and gastrectomy, related to alkaline reflux into the esophagus. To elucidate this correlation, the role of alkaline reflux of duodenal contents in the development of esophageal squamous cell carcinoma was investigated in Wister rats. Gastrectomized rats with regurgitation of duodenal contents into the esophagus were not administered any carcinogen and were sacrificed some at the end of 8 weeks and others at 50 weeks for pathological examination. Hyperplasia was found in rats at 8 weeks, and the esophageal squamous cell carcinoma was found in rats at 50 weeks. The carcinomas were found exclusively in the area of the reflux esophagitis and were accompanied by severe dysplasia. These results suggested that alkaline reflux of duodenal contents was strongly correlated to the development of the esophageal squamous cell carcinoma.

Key words total gastrectomy, squamous cell carcinoma, rat

INTRODUCTION

The reflux of duodenal juice is known as an important factor as carcinogenes is of remnant stomach cancer. Some reports have found that the frequency of lower esophageal cancer increased after total gastrectomy [1-3], and the cause of lower esophageal cancer after total gastrectomy may be related to reflux of duodenal juice [3]. However there have been few experiments about the effect of reflux of duodenal juice on carcinogenesis of lower esophageal cancer.

Here, we performed the total gastrectomy on Wister rats, and examined them at 8 weeks and 50 weeks after operation, to investigate the histological changes in the lower esophagus.

MATERIALS AND METHODS

Animals

Eight week-old male Wister rats were used as materials. They were fed standard maintenance diet and tap water without carcinogen.

Experimental procedure

Operative procedures were carried out after an intramuscular injection of 25 mg/kg of Nembutal. Total gastrectomy followed by esophago-jejunostomy was performed, in an attempt to induce regurgitation of duodenal contents consisting of bile and pancreatic juice into the esophagus. Drinking water was withheld for 24 hrs and food for 48 hrs after surgery. Thereafter, animals had free access to water and food.
Some at the end of 8 weeks and others after 50 weeks, the animals were sacrificed and all digestive organs were preserved by formalin fixation, embedded in paraffin, and stained with hematoxylin and eosin for histological examination.

Immunohistochemical staining

Sections of the esophagus 3 μm-thick were stained using the avidin-biotin-peroxidase technique (ABC method). In briefs deparaffinized tissue sections were immersed in methanol containing 0.3% H₂O₂ for 30 min to block endogenous peroxidase activity. The sections were then incubated with anti-PCNA antibody (diluted 1:500; DAKO, CA) for at least 12 hrs at 4°C, followed by incubation with biotinylated rabbit anti-mouse serum for 30 min and incubation with streptavidin-peroxidase complex for 30 min. Staining was developed by incubating the sections in 3-amino-9-ethylcarbazole (AEC) for 5 min. The sections were then counterstained in hematoxylin and mounted.

A total of 1000 cells was counted for each of the upper, middle and lower esophagus, and the average percentage of stained cells (PCNA Labelling Index (LI)) was calculated.

RESULTS

Macroscopic findings

At 8 weeks, the esophagus was abnormally thickened with a dilated lower portion. The mucosa of the lower esophagus was grossly irregular, with granular appearance (Fig. 1). At 50 weeks, the lower and
middle esophagus was abnormally thickened and dilated. The mucosa of the lower and middle esophagus was grossly irregular. The anastomotic portion was abnormally thickened and hard (Fig. 2).

**Histological findings**

At 8 weeks, severe esophagitis and erosion with abscess formation were observed from the lower esophagus to the anastomotic portion (Fig. 3). Squamous epithelium extended irregularly with hyperkeratosis observed in the lower esophagus histologically diagnosed as hyperplasia (Fig. 4). At 50 weeks, well-differentiated squamous cell carcinoma which invaded the submucosa was observed in the lower esophagus (Fig. 5). These malignant foci were intermingled with areas of esophagitis, hyperplastic lesions and severe dysplasia (Figs 6 and 7). No metastasis to lymph nodes or distant organs was observed. All the mucosal changes in the lower esophagus were histologically determined to be reflux esophagitis.

**PCNA labelling index (LI) (Table 1)**

At 8 weeks, the PCNA LI of the upper, middle, and lower esophagus was 3.70%, 5.77%, and 10.95%, respectively. At 50 weeks, the PCNA LI of the upper, middle, and lower esophagus was 7.24%, 14.80%, and 23.84%, respectively. The PCNA LI of the lower esophagus was higher than that in the upper, and than that in the middle esophagus, at 8 and at 50 weeks. The PCNA LI of the middle esophagus was higher than that of the upper esophagus at 8 and at 50 weeks. The PCNA LI at 50 weeks was higher than that at 8 weeks.

**DISCUSSION**

Since Druckey et al. [4] demonstrated that methylalkyl-nitrosamines specifically induced esophageal carcinoma, several investigators have reported that various experimental factors contributed to the development of esophageal carcinoma [5-7]. However
very few experimental studies have attempted to clarify the role of alkaline reflux of duodenal contents into the esophagus in the development of esophageal squamous-cell carcinoma. Togi [8] and Seto et al. [9] have reported in rats treated with N-amy1-N-methyl-Nitosamine (AMN), that alkaline reflux esophagitis acted as a promoter in the development of esophageal carcinoma. Barrett’s esophagus is thought to be a precancerous lesion of esophageal adenocarcinoma [10]. Barrett’s esophagus occurred from esophagitis by reflux [11]. Waring et al. [12] reported that reflux of duodenal contents into the stomach was seen in many patients with Barrett’s esophagus, and it was suggested that gastric or duodenal juice was a carcinogen of esophageal adenocarcinoma. Segawa et al. [13] investigated the role of reflux of gastroduodenal juice in the genesis of carcinoma in the esophagus and forestomach, and reported reflux of gastroduodenal juice induced not only chronic reflux esophagitis but also squamous cell carcinoma, Barrett’s esophagus, and mucinous adenocarcinoma in the esophagus and forestomach. Since 1984, the carcinogenic effects of bile on the gastric mucosa have been experimentally investigated [14]. Moreover, the regurgitation of duodenal contents or pancreatic juice alone into the stomach without any carcinogen has recently been observed to induce experimental gastric carcinoma [15,16]. A similar effect on the development of esophageal carcinoma was demonstrated in the present experiment.

In our experiment, rats which experienced regurgitation of the duodenal contents into the esophagus, developed esophageal carcinoma. Carcinomas were surrounded by mucosa damaged by alkaline reflux, and no carcinomas was observed in the upper esophagus, where the effects of alkaline reflux were not evident. These data confirmed that alkaline reflux of duodenal contents consisting of bile and pancreatic juice was correlated to the development of esophageal carcinoma in this animal model. At 8 weeks, esophagitis and hyperplasia were observed in the lower esophagus, and at 50 weeks the carcinomas were found exclusively in those areas of reflux esophagitis and were accompanied by severe dysplasia. These results suggested severe esophagitis occurred by reflux of duodenal juice and hyperplasia of basal cell layer from frequent destroying and regeneration of the squamous epithelium, at last dysplasia occurred from mutated stem cells, and squamous cell carcinoma developed from the severe dysplasia.

REFERENCES