Impact of Inflammation-Metaplasia-Adenocarcinoma Sequence and Prevention in Surgical Rat Models

Tomoharu Miyashita^a Furhawn A. Shah^d Koichi Miwa^b Shozo Sasaki^a
Koji Nishijima^a Katsunobu Oyama^a Itasu Ninomiya^a Sachio Fushida^a
Takashi Fujimura^a Takanori Hattori^c John W. Harmon^d Tetsuo Ohta^a

^aDepartment of Gastroenterologic Surgery, Kanazawa University Hospital, Kanazawa, ^bHouju Memorial Hospital, Nomi, and ^cDepartment of Pathology, Shiga University of Medical Science, Otsu, Japan; ^dDepartment of Surgery, Johns Hopkins Bayview Medical Center, Johns Hopkins University School of Medicine, Baltimore, Md., USA

Key Words

Histogenesis · Carcinogenesis · Barrett's esophagus · Esophageal cancer · Esophageal adenocarcinoma · Chemoprevention

Abstract

The incidence of esophageal cancer continues to rise in the Western world. Prior studies have suggested that gastroduodenal content reflux from gastroesophageal reflux disease induces the inflammation-mediated progression from hyperplasia to metaplasia, and to adenocarcinoma. We further investigated the sequential development of esophageal adenocarcinoma (EADC) with the use of an established surgical rat model. The present paper will describe the impact of the inflammation-metaplasia-adenocarcinoma sequence and chemoprevention in surgical rat models. A clinically relevant rat reflux model was used to investigate the cause of carcinogenesis, the sequential development of adenocarcinoma and chemoprevention with the use of a proton pump inhibitor. We found that duodenal reflux plays an important role in the inflammation-induced transformation of esophageal mucosa to adenocarcinoma. We were able to inhibit this transformation with rabeprazole, a proton pump inhibitor. Duodenal reflux promotes inflammation in the esophagus.

The inflammation-metaplasia-adenocarcinoma sequence is important in the progression and development of EADC. Carcinogenesis can be prevented with chemoprevention agents such as rabeprazole. These results will need to be validated in clinical trials.

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Introduction

The incidence of esophageal cancer continues to rise in the Western world [1, 2]. The cause of the increase in incidence remains obscure and has prompted further investigation into this clinical problem. Prior studies have proposed that esophageal adenocarcinoma (EADC) develops through a sequence of events. Specifically, gastroduodenal content reflux from gastroesophageal reflux disease (GERD) induces the inflammation-mediated hyperplasia and metaplasia. This results in dysplasia and EADC [3]. The change in the diet and lifestyles in the Western world has contributed to an increase in the prevalence of obesity and hiatal hernias, and an increase in GERD. These lifestyle changes are thought to be responsible for the rise in the incidence of esophageal cancer [4].

Fig. 1. Diagram of the surgical procedure. The DGER model promotes regurgitation of both duodenal and gastric juice. The DER model produces regurgitation of duodenal juice alone. The GER model promotes regurgitation of gastric juice alone. The RY procedure does not result in duodenal or gastric regurgitation. The incidence of esophageal carcinoma (Ca.) in the DGER or the DER was significantly higher than in the GER or the RY ($p < 0.001$). Esophageal carcinoma developed only in models with duodenal refluxate. No exogenous carcinogen was administered in this experiment.

We investigated the sequential development of EADC using a surgical rat model. Prior studies have determined that duodeno-esophageal (DER) or duodeno-gastro-esophageal reflux (DGER) in a rat model induced EADC [5, 6]. These cancerous changes occurred without the use of any exogenous carcinogens. The rat model confirmed the histopathological sequence of events from GERD to EADC. Furthermore, a recent study established a relationship between the amount of reflux and the likelihood of developing EADC. The present paper will expand on prior reviews [7]. The goal is to describe the impact of the inflammation-metaplasia-adenocarcinoma sequence and chemoprevention in clinically relevant surgical rat models.

Duodenal Content Reflux Induces Esophageal Carcinogenesis

We established a surgical rodent model in which the forestomach was anastomosed with jejunum [5]. This duodeno-forestomach or duodeno-glandular-forestomach reflux model promoted gastro-duodenal reflux. The rodents subjected to this intervention developed squamous cell carcinoma, columnar epithelial metaplasia and mucinous adenocarcinoma. This was achieved without the use of carcinogens. Subsequent studies have confirmed these findings with alternate rodent models [3, 8].

The initial study suggested that duodenal reflux alone was sufficient to induce esophageal carcinogenesis [6, 8, 9]. Other studies reported that a combination of gastric and duodenal contents was required to produce carcino-

genesis. The importance of gastric reflux alone remained unclear.

This was further investigated with the development and use of four distinct rodent surgical models to determine the component responsible for carcinogenesis [6]. The DGER model promotes the reflux of both gastric and duodenal contents. The DER model consists of a total gastrectomy and anastomosis of the esophagus to the jejunum, thus producing reflux of duodenal contents alone. The gastro-esophageal reflux (GER) model includes the transposition of the duodenum distally. The model promotes gastric reflux alone. A Roux-en-Y (RY) procedure was developed to be used as a control with no reflux induction. Rodents in the duodenal reflux groups, DGER and DER, were the only rodents to develop esophageal carcinoma. The rodents not subjected to reflux or gastric reflux alone did not develop esophageal carcinoma (fig. 1). These findings have been confirmed by other subsequent studies. Furthermore, the study suggested that gastric juice may even be protective, since fewer rodents developed EADC in the DGER group than the DER group [10]. This may be secondary to alteration of the carcinogenic effect of bile compounds with exposure to an acid milieu.

We have established that duodenal reflux is important in the development of EADC. More recently, we concluded that there is an association between the severity of DER and the histological type of esophageal cancer. We found that increased duodenal content reflux promoted the development of EADC, while rodents subjected to mild duodenal content reflux were more likely to develop esophageal squamous cell carcinoma [11].

Fig. 2. Microscopic findings of DER in rat. **a** Metaplasia of the gastric foveolar epithelium was observed in the basal layer of the squamous epithelium, indicated by arrows. **b** Pyloric gland metaplasia with abundant mucus extending from the gastric foveolar epithelial metaplasia to the basal layer, indicated by arrows. **c** Completed columnar-lined metaplasia covered the entire mucosal layer, indicated by arrows, which is called Barrett's esophagus. Serial sections stained with GOS (**d**), Con A (**e**) or HID-AB (**f**). In addition to gastric foveolar epithelial and pyloric gland metaplasia, intestinal epithelial metaplasia was observed. **g, h** Representative photomicrographs of esophageal carcinoma that resulted from surgically induced gastroduodenal reflux: adenocarcinoma (**g**), squamous cell carcinoma with keratin pearls (**h**).

Inflammation-Metaplasia-Adenocarcinoma Sequence in a Rat Model

A rodent surgical reflux model was developed to further investigate the pathogenesis of esophageal cancer [8]. The DER model led to the exposure of the esophageal mucosa to duodenal contents. This exposure resulted in an inflammatory response. The animals were sacrificed

post-surgically at 10-week intervals for a total of 50 weeks. Within 10 weeks of surgery, the squamous epithelium was infiltrated with lymphocytes. There were signs of proliferative squamous hyperplasia, regenerative thickening and basal cell hyperplasia. In 20 weeks, glandular structures were seen throughout the basal layer of the esophageal epithelium. This was followed with proliferative squamous hyperplasia and erosions near the anasto-

Table 1. Histopathological findings

	Placebo group	Rabeprazole group
Total surgeries	38	38
Survivors	23	17
Average weight $\pm$ SE, g	292 $\pm$ 58	265 $\pm$ 43
Cancer	17 (74%)	5 (29%)
Adenocarcinoma	14	5
Squamous cell carcinoma	3	2
Barrett's metaplasia	23 (100%)	11 (65%)
Proliferative hyperplasia	23 (100%)	17 (100%)

The incidence of cancer ($p < 0.05$, χ^2 test) and Barrett's metaplasia ($p < 0.05$, Fisher's exact test) was lower in the rabeprazole group as compared to the placebo group. Two animals developed both squamous cell carcinoma and adenocarcinoma.

quence of several events progressing from DEGR to EADC is thought to involve the development of inflammation-stimulated proliferative squamous hyperplasia and metaplasia such as in Barrett's esophagus, followed by multifocal and EADC (fig. 3).

Chemoprevention for Barrett's Metaplasia and Adenocarcinoma

We have established that significant bilious reflux increases the likelihood of EADC in a rodent reflux model. This model allowed us to investigate the response to chemopreventive agents.

We evaluated the effectiveness of the proton pump inhibitor rabeprazole as a chemoprevention agent in a surgical rat reflux model of esophageal cancer [18]. We found that rabeprazole significantly reduced the incidence of Barrett's metaplasia and esophageal cancer (table 1). We hypothesized that the proton pump inhibitor raised pH and altered the interactions between duodenal and gastric milieu. This led to the production of substances that promoted inflammatory changes. These substances may be bile acids altered by the milieu or even pancreatic enzymes. Alternatively, rabeprazole may possess anti-inflammatory properties that protect the esophageal mucosa.

Prior epidemiological studies revealed long-term use of proton pump inhibitors decreased the incidence of dysplasia and adenocarcinoma in patients with an established diagnosis of Barrett's esophagus [19, 20]. However, the data remains mixed. Another large review compared

the efficacy of anti-reflux surgery with chemotherapy in the prevention of progression of Barrett's esophagus to EADC. The study found that surgery decreased the likelihood of progression of Barrett's esophagus to EADC and increased the likelihood of regression of Barrett's esophagus [21]. A more recent trial to evaluate the effects of omeprazole in combination with aspirin on the rate of progression to high-grade dysplasia or EADC in patients with Barrett's esophagus is ongoing. Preliminary results suggest a synergistic anti-inflammatory effect [22].

Conclusions

The use of a clinically relevant surgical rodent reflux model allowed us to better understand the pathogenesis of esophageal adenocarcinoma. The model was used to verify the importance of duodenal contents reflux in the promotion of esophageal cancer. Inflammation was found to be the key component of the transformation of the esophageal mucosa to adenocarcinoma. Chemoprevention with rabeprazole was suggested to provide an anti-inflammatory and protective effect. While the results obtained from our animal model are impressive, they require further investigation and verification with human clinical trials.

Disclosure Statement

The authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

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