Effects of refluxate pH values on duodenogastroesophageal reflux-induced esophageal adenocarcinoma

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Abstract

AIM: To determine the effects of duodenogastric juice pH on the development of esophageal adenocarcinoma (EAC).

METHODS: An animal model of duodenogastroesophageal reflux was established using Sprague-Dawley (SD) rats undergoing esophagoduodenostomy (ED). The development of EAC was investigated in rats exposed to duodenogastric juice of different pH. The rats were divided into three groups: low-pH group (group A), high-pH group (group B) and a sham-operated group as a control (group C) (n = 30 rats in each group). The incidence of esophagitis, Barrett’s esophagus (BE), intestinal metaplasia with dysplasia and EAC was observed 40 wk after the treatment.

RESULTS: The incidence rate of esophagitis, BE, intestinal metaplasia with dysplasia and EAC was higher in groups A and B compared with the control group after 40 wk (P < 0.01), being 96% and 100% (P > 0.05), 88% and 82.4% (P > 0.05), 20% and 52.1% (P < 0.05), and 8% and 39% (P < 0.05), respectively.

CONCLUSION: Non-acidic refluxate increases the occurrence of intestinal metaplasia with dysplasia and EAC while the low-pH gastric juice exerts a protective effect in the presence of duodenal juice. The non-acid reflux is particularly important in the progression from BE to cancer. Therefore, control of duodenal reflux may be an important prophylaxis for EAC.

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Key words: Esophageal reflux; Esophageal adenocarcinoma; pH-metry; Pathogenesis

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INTRODUCTION

The incidence rate of esophageal adenocarcinoma (EAC) has been increasing more rapidly than that of other malignancies[1]. This rapid increase may be related to the increasing occurrence of gastroesophageal reflux disease (GERD) and Barrett’s esophagus (BE)[2]. BE is the main risk factor and an acquired condition for these tumors[3]. Gastric acid has been regarded as the major risk factor for GERD, and acid suppression is the first-line treatment[4]. However, the role of the gastric acid in the development...
of GERD remains controversial.

Gastric juice refluxing into the esophagus contains gastric, biliary and pancreatic secretions that have refluxed into the stomach from the duodenum. Early studies showed that reflux of combined duodenal and gastric juices into the esophagus caused severe esophagitis, and reflux of duodenal juice alone resulted in a similar degree of esophageal injury. It has been shown that esophageal exposure to duodenal juice is a key factor in the genesis of BE and EAC. Some researchers have suggested that the obvious increase in the incidence of EAC might be related to the acid suppression. However, to dynamically monitor the duodenal juices and clarify the role of duodenal juice reflux in the pathologic process has attracted much attention. Some studies have confirmed that duodenal juice reflux could induce BE and EAC in rats.

Improved animal models are therefore needed to examine the role of non-acidic reflux in EAC induced by duodenal juice reflux in the absence of exogenous carcinogens. The aim of this study was to investigate the roles of gastric and duodenal juices in the genesis of EAC in a rat model.

MATERIALS AND METHODS

Experimental animal

Ninety 8-wk-old Sprague-Dawley (SD) rats weighing 200-250 g were purchased from the Experimental Animal Center of Xi’an Jiao Tong University. The male pairing female rats were randomly divided into three groups, each with 30 rats.

Animal model

A rat model of duodenogastroesophageal reflux, with different pH values of reflux contents, was established in accordance with the method of Zhang et al. Surgical diversion of duodenal secretions into the esophagus in the experimental group was induced by end-to-side esophagoduodenostomy (ED). The rats were divided into a low-PH group (group A) and a high-PH group (group B), with 30 rats in each group. A sham-operated group (group C, n = 30) was used as a control group (Figure 1). The esophagus was separated from the posterior vagal trunk and left gastric vessels, tied with silk at the gastroesophageal junction, and dissected 2 mm proximal to the tie. The anterior vagus nerve was protected from damage when the esophagus was cut with 16 interrupted stitches of 7-0 polypropylene. The purpose of the anastomosis was to induce the reflux of both gastric and duodenal juices into the esophagus. In group A, the anterolateral wall of the duodenum at the distal end 1 cm from the pylorus was opened longitudinally, and anastomosed with the cut end of the esophagus. In group B, the anterolateral wall of the duodenum at the distal end 2 cm from the pylorus was opened longitudinally and anastomosed with the cut end of the esophagus. In group C, the lower esophagus and the first portion of the duodenum were dissociated. Surgery was performed after an acclimatization period of 4 d. Rats were kept in hanging cages under a 12 h light-dark cycle at a temperature of 21°C and a humidity of 60%. Water and standard chow were provided ad libitum. Food was discontinued the evening before surgery, and water was discontinued on the morning of surgery. Rats were anesthetized with an intramuscular injection of xylazine hydrochloride (18 mg/kg) and ketamine (72 mg/kg), with further doses administered intraperitoneally during surgery, as required. Before closure, 0.5-1.5 mL of 0.9% sodium chloride was instilled into the peritoneal cavity. Water was permitted when the rats awoke, and chow was provided the next day. The rats were housed in cages at 22-25°C with free access to standard rat pellet food and water for 40 wk. Rats were treated following the guidelines for the care and use of laboratory animals of the National Animal Welfare Committee.

Intraluminal pH was measured using a pH glass electrode of Digitrapper MK Portable pH Monitor (Sweden Medtronic Synectics Company, Stockholm, Sweden). It was positioned in the distal end of the esophagus, the forestomach, the opisthogaster and the duodenum 1 and 2 cm from the pylorus in the process of esophagoduodenostomy. It was also measured after rats were killed 40 wk after operation.

Tissues and specimens

Rats were killed 40 wk after operation. The esophagus was opened longitudinally and gross pathologic changes were observed. Esophagitis, BE and EAC were differentiated, and samples of the three abnormal tissues (0.2 × 0.2 cm²) were removed, and fixed in formalin. Paraffin sections were stained with hematoxylin-eosin and observed under a light microscope.

Statistical analysis

The incidence rates of esophagitis, BE and EAC between the groups were analyzed and compared using χ² tests with SPSS software, and differences in numerical data were compared between groups using t test. The level of significance was set at P < 0.05.

RESULTS

The number of the surviving rats in the three groups was 25, 23 and 29, respectively. The overall mortality was 14.4%.
pH values in different parts of esophagus, stomach and duodenum

The pH values increased from the proventriculus down to the duodenum 2 cm away from the pylorus ($P < 0.05$), (Figure 2). The preoperative pHs at the distal end of the esophagus in groups A and B were significantly lower than the postoperative values ($P < 0.05$).

Postoperative pH values in the distal esophagus in groups A and B

The pH value in the distal esophagus in group A, in which the duodenum was cut 1 cm from the pylorus, was 6.14 ± 0.36, which was significantly lower than that in group B (8.27 ± 0.46, $P < 0.01$). There was no significant difference in preoperative pH values in the distal esophagus between the two groups ($P = 0.12$).

Gross observations

In the sham-operated group, the esophageal wall was thin, with a smooth mucosa, and the esophageal lumen was uniform in size along its length. Blood vessels were visible below the mucous membrane, and congestive inflammation was occasionally visible in the distal esophagus. In most animals in both groups, the lumen of the middle and the lower parts of the esophagus was dilated. Esophagitis appeared as mucosal hyperplasia, with a thickened, rough surface with small and large kernels in longitudinal rows, becoming less pronounced from the distal to the proximal end. Hyperemia, edema, mild erosion and indistinct blood vessels below the mucous membrane were visible at the proximal end. BE occurred mostly in the distal esophagus at the stoma between the esophagus and duodenum, and appeared as an unclear boundary between the esophagus and the duodenal mucosa. The esophagus was inflamed at the proximal end, smooth and velvety, with a clear boundary from the duodenum. The area of BE generally extended for about 0.5-2 cm, with chronic proliferation and inflammation at the proximal end of the esophageal mucosa. Small sheets of BE pathology were seen in some cases of esophagitis. The upper esophagus was normal in BE, and all esophageal adenocarcinomas (EACs) developed near the proximal end of the stomach in BE, with nodular hyperplasia, ulcer and fish-like appearance. The esophagus at the upper end of the tumor was obstructed, with obvious dilatation and changes in the features of BE. The hyperplasia was reduced at the proximal end of the obstruction, and appeared as congestion of 1-2 mm and presented edema changes (Figure 3).

Histologic characteristics

Normal esophageal epithelium appeared as stratified squamous epithelium, with neat rows, some showing keratinization. Esophagitis appeared as hyperplasia of the stratum epithelial basal cells, excessive keratinization and papillomatosis, visible neutrophilic granulocytes, infiltrated lymphoepithelial cells, mucosal erosion and edema of the submucosa in the mucosa and the lower layers of the muscosa. BE
recent research has shown that reflux of both gastric and esophageal adenocarcinoma (EAC) has been the subject of numerous studies. The evidence rate of gastroesophageal reflux disease (GERD) reflects the increasing incidence rate of other tumors. The incidence rate of EAC has increased significantly in recent years, more rapidly than that of the esophagus. GERD occurs when the contents of the stomach and duodenum are regurgitated into the esophagus, causing pathologic lesions of the mucosa, and pathologic changes in the esophagus. The mechanism that gastroesophageal reflux induces duodenal juices can damage the esophageal mucosa. However, the contributions of the specific components of gastroesophageal reflux to the development of EAC remains unclear. The current study used an animal model, in which the pH values of the duodenogastric reflux could be varied, to investigate the effects of gastric acid and duodenal juice on the EAC induced by gastroesophageal reflux, with the aim of identifying the specific responsible factors.

Gastric acid is believed to be an important contributory factor in reflux esophagitis and Barrett esophagus. Intestinal metaplasia with dysplasia and EAC showed replacement of the squamous mucosa with simple columnar epithelium. Intestinal metaplasia with dysplasia showed replacement of the mucosa with simple columnar epithelium, changes in the size and shape of hyperplastic cells, with large, hyperchromatic nuclei and increased nucleoplasm, irregular arrangement of the cells, disappearance of cell polarity, and irregular shape and arrangement of the glandular cells. However, these changes were not characteristic of cancer and no obviously abnormal cells could be seen and no pathologic invasion to the basilar membrane. EAC showed severe intestinal metaplasia with dysplasia, pathologic invasion to the basilar membrane, and some to the blood or lymphatic vessels (Figure 4).

### Incidence rates of esophagitis, BE, intestinal metaplasia with dysplasia, and EAC

The incidence rate of esophagitis in groups A and B 40 wk after the treatment was 96% and 100%, respectively ($\chi^2 = 0.930, P = 0.330$). The incidence rate of Barrett esophagus (BE) was 88% and 82.4%, respectively ($\chi^2 = 0.280, P = 0.60$), and of intestinal metaplasia with dysplasia was 20% and 52.1%, respectively ($\chi^2 = 5.420, P = 0.02$). The incidence rate of EAC was 8% and 39% in the two groups, respectively ($\chi^2 = 6.570, P = 0.01$). All these rates were significantly higher than in the sham-operated control group ($P < 0.001$) (Figure 5).

### DISCUSSION

GERD occurs when the contents of the stomach and duodenum are regurgitated into the esophagus, causing pathologic lesions of the mucosa, and pathologic changes in the esophagus. Gastroesophageal reflux can result in the development of EAC. The incidence rate of EAC has increased significantly in recent years, more rapidly than that of other tumors, and the annual increase in the incidence rate of GERD reflects the increasing incidence rate of EAC. Clinical epidemiological studies have shown that gastroesophageal reflux correlates closely with EAC.

The mechanism that gastroesophageal reflux induces EAC has been the subject of numerous studies, and a recent research has shown that reflux of both gastric and duodenal juices can damage the esophageal mucosa. However, the contributions of the specific components of gastroesophageal reflux to the development of EAC remains unclear. The current study used an animal model, in which the pH values of the duodenogastric reflux could be varied, to investigate the effects of gastric acid and duodenal juice on the EAC induced by gastroesophageal reflux, with the aim of identifying the specific responsible factors.

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ferred depending on the position in the esophagus relative to the anastomosis of the esophagus and duodenum; the pH value 2 cm away from the pylorus was higher than that 1 cm away from the pylorus ($P < 0.01$).

These results confirm that the esophagus was stimulated by the contents of the stomach and duodenum with different pH values; a higher pH indicated a higher proportion of duodenal juice, while a lower pH indicated a higher proportion of gastric juice.

Esophagitis, BE, intestinal metaplasia with dysplasia and EAC developed in both the treated groups after 40 wk. The incidence rates of intestinal metaplasia with dysplasia and EAC were higher in the high-pH group, compared with the low-pH group ($P < 0.01$). There were no significant differences in the incidence rate of esophagitis or BE.

The results of this study showed that the reflux of gastric juice and duodenal contents could induce EAC in rats. More acidic duodenogastric reflux was associated with lower incidence rate of intestinal metaplasia with dysplasia and EAC, compared with more basic duodenogastric reflux. These results suggest that duodenal juice reflux increases the incidence rate of intestinal metaplasia with dysplasia and EAC, thus playing an important role in the pathogenesis of EAC, while gastric juice reflux was null or had no significant effect. The results imply that non-acid reflux is particularly important in the progression from BE to cancer. Therefore, control of duodenal reflux may be an important prophylaxis of the EAC.

**COMMENTS**

**Background**

The incidence rate of esophageal adenocarcinoma (EAC) is rising faster than that of any other cancers. Clinical epidemiological studies have shown that gastroesophageal reflux correlates closely with EAC. However, the relationship between the specific reflux components and the induction of EAC remains unclear.

**Research frontiers**

Gastroesophageal reflux can cause EAC, and the mechanisms have been the subject of extensive research. The specific gastroesophageal reflux components responsible for EAC remain largely unknown. In this study, the authors demonstrated that non-acid reflux increases the incidence rates of intestinal metaplasia with dysplasia and EAC, while acidic reflux had an opposite effect.

**Innovations and breakthroughs**

Recent reports have highlighted the importance of duodenal juice in the pathogenesis of EAC. The duodenum contains bile, pancreatic juice, and intestinal juice, of which cholic acid, trypsin and hemolytic lecithin could damage the esophageal mucosa. This is the first study to report a relationship between the pH of the duodenogastric refluxate and the incidence of EAC. The results of this study therefore suggest that duodenal juice plays an important role in the pathogenesis of EAC and gastric juice had an opposite effect.

**Applications**

Better understanding of the roles of the specific esophageal reflux components in the pathogenesis of EAC may represent a future strategy for the prevention of EAC.

**Peer review**

The article is original and well-thought. The topic of the research is important, as it would add to the body of evidence regarding the role of alkaline reflux in esophageal carcinoma. The manuscript is clearly laid out and well written. The methodology/design is suitable to answer the questions posed.

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