

Effect of Pancreatic Juice and Bile Reflux to the Development of Esophageal Carcinogenesis in Rat Model

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2. Keywords

Pancreatic Juice; Bile acid;
Esophageal Cancer; Reflux of
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1. Abstract

1.1. Aim: Reflux of duodenal contents contributes to the development of esophageal mucosal lesion. Esophageal cancer after total gastrectomy has been associated with the reflux of duodenal content (biliary and pancreatic juice) into the esophagus. This study is to determine which fraction of the duodenum content, reflux, pancreatic juice or bile acids contributes to the development of esophageal cancer.

1.2. Methods: 8 week Wistar Rat were used. 1. Reflux of Pancreatic juice and Bile (TG): End-to-end esophago-duodenostomy with total gastrectomy(n=27) was performed to produce pancreatic juice and bile reflux. 2. Reflux of Pancreatic Juice (TG+B): End-to-end esophago-duodenostomy with total gastrectomy. Then, a bypass operation of the upper bile duct was made 25cm below the esophagoduodenostomy anastomosis to produce only pancreatic reflux. Choledochojejunostomy was performed. (n=12) 3. Sham group (n=5). Forty weeks after operation, all rats were euthanized and the esophagus was evaluated histologically. Esophageal injury was evaluated by macroscopic and microscopic findings.

1.3. Results: 1. Macroscopic finding: In TG rats, the esophageal wall was thickened and severe inflammation. There was a small polypoid tumor in the lower esophagus in TG. The tumor is SCC and ADC. But TG+B was not severe inflammation, moreover the esophagus of TG+B did not reveal any pathological findings. 2. Microscopic findings: TG showed histological features of esophagitis including marked hyperplastic changes with increased thickness of squamous epithelium, hyperkeratosis and regenerative changes with papillomatosis and basal cell hyperplasia. By the way, TG+B showed slightly dysplasia of the esophagus. In TG, we detect erosion (100%), regenerative hyperplasia (100%), CLE (40%), severe dysplasia(100%),SCC(40%) and ADC(30%). In TG+B, we detect erosion(0%),regenerative hyperplasia(100%),CLE(0%),mild dysplasia(40%),SCC(0%) and ADC(0%).

1.4. Conclusion: The reflux of pancreatic juice alone is probably not significant development of esophageal cancer after total gastrectomy compared to the reflux of bile and pancreatic juice. Pancreatic juice reflux appears to exert a co-carcinogenic effect when combined with bile.

3. Introduction

Recently, the result of surgical treatment for gastric cancer improved. We have many cases of long survivor in gastric cancer. The incidence of esophageal cancer patients who have undergone distal gastrectomy is increasing recently [1]. The etiology of post-gastrectomized esophageal cancer is the regurgitation of gastric and duodenal contents into the esophagus. The relationship be-

tween gastrectomy and the subsequent development of esophageal cancer remains controversial. Animal studies [2,3] have shown that gastroesophageal reflux can increase the incidence of esophageal squamous cell carcinoma, even without the administration of any carcinogen. Miwa et al. [4] performed three types reflux model, Gastro-Duodenal-Esophageal Reflux (GDER), Duodeno-Esophageal Reflux (DER) and Gastroesophageal Re-

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flux (GER) to explain the relationship between refluxed duodenal contents and esophageal carcinoma.

They detect esophageal cancer, GER 0/16 (0%), GDER 10/12 (83%) and DER 10/13 (77%). They concluded refluxed duodenal contents are important factor to induce esophageal carcinogenesis and gastric acid is not important factor. We also performed rat model, esophagoduodenal anastomosis with total gastrectomy in order to produce reflux esophagitis. After 40 weeks of reflux, dysphagia 100%, Squamous Cell Carcinoma (SCC) 40%, adenocarcinoma (ADC) 30% [5].

Duodenal juice contains bile, pancreatic juice. It is not clear which component or combination of these components plays a role in producing ADC and SCC. In the present study, esophageal neoplasms were induced in a rat esophageal reflux model to investigate the effects of the different components of duodenal juice in the production of esophageal tumors. To simplify the interpretation of results, acid reflux was prevented in all rats.

4. Material and Methods

Eight week old Wistar Rats weighing 200-250g were used. The animal care and use committee of Kindai University prospectively approved all procedures.

4.1. Surgical Procedure

The rats were permitted to acclimate for 2 weeks before surgery. Prior to surgery, the animals were fasted for 24 hours. An esophago-duodenal anastomosis and choledochojejunostomy were performed under general anesthesia (somnolently 50 mg/kg body weight intraperitoneal injection) through an upper middle incision (Figure 1)

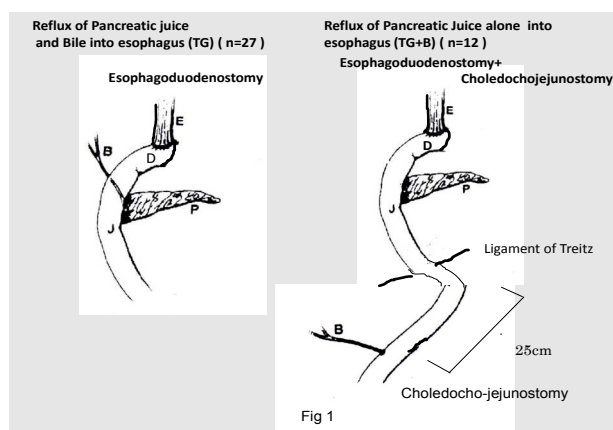


Figure 1: Surgical Procedure TG, TG+B.

Reflux of pancreatic juice and bile (TG): End-to-end esophago-duodenostomy with total gastrectomy (n=27) was performed to produce pancreatic juice and bile reflux. The gastroesophageal junction was ligated and the distal esophagus was transected 2mm above the ligature. Moreover, the gastroduodenal junction was also ligated, and the proximal duodenum was transected 3mm distal to the pylorus. A total gastrectomy was performed with the removal of the entire stomach, and end-to-end anastomosis of the esophagus and duodenum.

Reflux of pancreatic juice (TG+B): End-to-end esophago-duodenostomy with total gastrectomy. Then, a bypass operation of the upper bile duct was made 25cm below the ligament Treitz to produce only pancreatic reflux. Choledocho-jejunostomy was performed (n=12). The technique of biliary duct implantation in the small bowel was done as follows. The bile duct was exposed, divided and ligated distally with two 5-0 Vicryl ligatures. A vinyl chloride tube (length 30mm, external diameter 0.61mm, internal diameter 0.28mm, Becton Dickinson and Company) was used as a stent. One end of the tube was inserted into the divided CBD 5mm distal to the hepatic confluence and ligated in place with 5-0 Vicryl (Figure 2 A). The other end was inserted into 18G needle which was inserted through the jejunum from the mesenteric side to the antimesenteric side of the jejunum lumen 25 cm distal to the ligament of Treitz (Figure 2B). After the 18G needle was removed from the jejunum, proximal end of the tube held in place with a purse-string suture with 5-0 nylon, which was ligated with 5-0 Vicryl in common bile duct (Figure 2C). The ligated CBD tube brought out through the mesenteric side of the jejunum was cut just enough to re-establish bile flow. The cut end spontaneously reacted into the intestinal lumen. And the hole in the jejunum was closed with a stitch using 5-0 nylon (Figure 2D). Sham group (n=5) Control. Forty weeks after operation. All rats were euthanized and the esophagus was evaluated histologically. Esophageal cancer and dysplasia were evaluated by macroscopic and microscopic findings. Moreover, at the time of autopsy, blood was drawn for liver function. Serum levels of total bilirubin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) were determined using standards auto analyzer method (Hitachi Auto analyzer 736; Hitachi Tokyo ,Japan).

4.2. Statistics

Comparisons between groups were made by the Mann-Whitney test. A difference between groups of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using the Stat View 5.0-J program (Abacus Concepts, Berkeley, CA, USA)

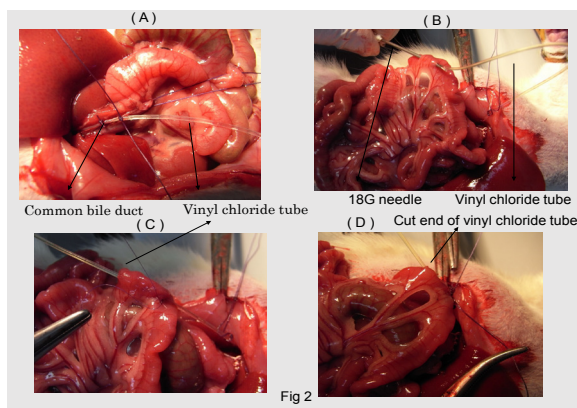


Figure 2: Procedure of Choledcho-jejunostomy.

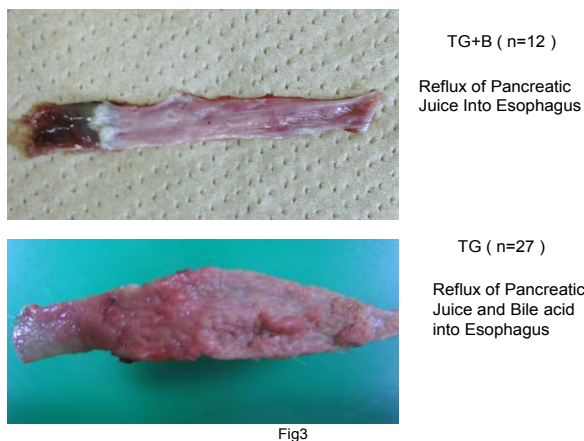


Figure 3: Macroscopic finding.

5. Result

Macroscopic finding (Figure 3):

The middle and lower esophagus of animals in TG was wide thickened and sever inflammation. There was a small polypoid tumor in the lower esophagus in TG. But TG+B was not sever inflammation. Moreover, the esophagus of TG+B did not reveal any pathological findings.

Microscopic finding (Figure 4, Figure 5): TG group showed histological features of esophagitis, including marked hyperplastic changes with increased thickness of squamous epithelium, hyperkeratosis and regenerative changes with papillomatosis and basal cell hyperplasia. By the way, TG+B showed slightly dysplasia of the esophagus. We detected 100% erosion, 100% regenerative hyperplasia, 40% CLE, 100% sever dysplasia, 40% SCC and 30% ADC in TG (n=27). By the way, we detected 0% erosion, 100% regenerative hyperplasia, 0% CLE, 40% mild dysplasia, 0% SCC and 0% ADC in TG+B (n=12).

Serum biochemical examination (Table 1): There is no difference between control and TG+B rat in T-Bil, Alp. But AST and ALT in TG+B are higher compared to those of control.

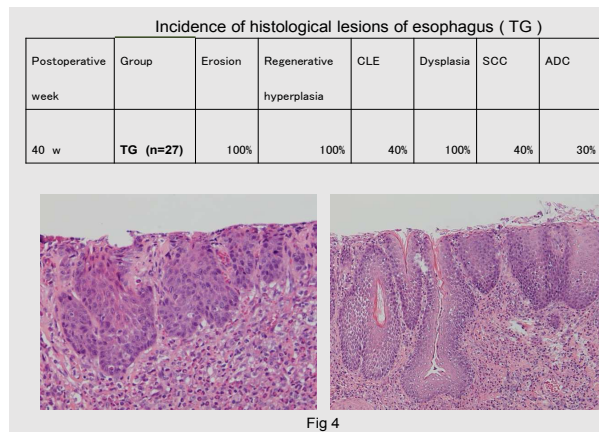


Figure 4: Incidence of histological lesions of esophagus and microscopic finding in TG.

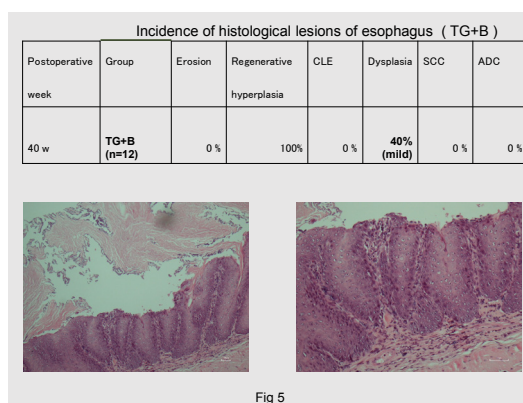


Figure 5: Incidence of histological lesions of esophagus and microscopic finding in TG+B.

Table 1: Serum biological examination.

	Control	TG+B
Total Bil mg/dl	0.8±0.2	1±0.2
AST IU/L	96±5	225±30*
ALT IU/L	40±14	85±18*
Alp IU/L	217±15	115±70

*P<0.05

6. Discussion

In this study, it was found that with the esophageal reflux of pancreatic juice together with bile, there was promotion of the appearance of SCC and ADC, but reflux of pancreatic juice alone did not have this effect. In animal studies it has been reported that both bile and pancreatic juice can cause esophageal cancer, whereas gastric juice has less of an effect [6]. It is interesting theme which component of duodenal contents, bile or pancreatic juice causes esophageal carcinogenesis. From the serum biochemical examination, serum bilirubin, and Alp in TG+B was similar to those of control.

Therefore, there is no problem about the bile flow via choledcho-jejunostomy in TG+B.

6.1. Bile acid

Bile is present in greater concentration in the stomach and esophagus of patients with severe esophagitis or Barrett's esophagus [7,8]. There is a high incidence of gastric and esophageal cancer in patients after gastrectomy, in which free reflux of duodenal content into the stomach and esophagus occurs [9,10]. Moreover, individuals with esophageal adenocarcinoma experience even greater exposure to bile than persons with uncomplicated BE [11]. Expression of bile acid transporter proteins is increased in BE tissues, suggesting that the development of BE metaplasia may be an adaptation to protect cell from bile acids [12]. Thus progression to BE and to adenocarcinoma may be strongly influenced by bile acid exposure. Evidence indicates that short-term exposure of esophageal cells to bile acids induces oxidative stress, DNA damage, mutation and apoptosis. Therefore, bile acids are implicated as etiologic agents in cancer of the gastrointestinal tract, including cancer of the esophagus and stomach.

6.2. Pancreatic juice

Pancreatic juice contains trypsin, chemotrypsin, phospholipase A2 and other digestive enzymes. Overexpression of trypsin has been implicated in tumor growth invasion and metastasis. Yamamoto et al. [13] clarify the clinicopathologic and prognostic significance of trypsin expression in SCC. Trypsin positivity was significantly correlated with the depth of invasion, lympho node metastasis, advanced pTNM classification, recurrence. They concluded trypsin plays a key role in the progression of esophageal carcinoma. Yamashita et al. [14] performed rat models: diversion of bile alone, pancreatic juice alone, both bile and pancreatic juice into the esophagus. Two weeks after surgery, rats were treated with the esophageal carcinogen, 2,6-dimethylnitrosomorpholine 48mg/kg ip weekly for 20 weeks. The rats were killed at age 30 weeks. The prevalence of DNA aneuploidy and histologic esophageal papillomatosis or SCC were increased in carcinogen treated rats with pancreatic juice reflux and the combination of pancreatic and bile reflux but not in rats with bile reflux alone. They concluded that pancreatic juice was the most potent component of the duodenal refluxate in the promotion of esophageal carcinogenesis in rats.

Pera³ was devised to investigate the influence of pancreatic and biliary duodenal content reflux on the induction of esophageal carcinoma. 2,6-dimethylnitrosomorphine was injected subcutaneously weekly from 2 week to 32 week. Carcinoma of the esophagus was induced only in rats receiving the carcinogen after exposure to either pancreatic reflux (3/22, 13%) or pancreatic and biliary reflux (9/27, 33%). In contrast, no carcinomas were observed in the groups with carcinogen plus biliary reflux alone. They concluded pancreatic juice and pancreaticobiliary secretions had more effect than bile in promoting esophageal carcinogenesis induced by 2,6-dimethylnitrosomorphine.

Since trypsin in alkaline duodenal contents is a major injurious enzyme to esophageal mucosa, pancreatic juice might modify and promote the esophageal carcinogenesis.

But in our data, esophageal cancer was detected in pancreatic juice alone reflux (0%), pancreatic and biliary reflux (SCC 40%, ADC 30%) without carcinogen. Busby et al. [15] reported that some N-nitroso-bile acids, such as N-nitrosotaurocholic acid and N-nitrosoglycocholic acid, act not only as mutagens but also as carcinogens. Mirrish et al. [16] reported that bile acid in the refluxate can be a source of amides which react with nitrite, producing carcinogenic N-nitrosamides. Therefore, bile is important esophageal carcinogenic factor. Pancreatic juice reflux appears to exert a co-carcinogenic effect when combined with bile.

7. Conclusion

The reflux of pancreatic juice alone is probably not significant development of esophageal cancer after total gastrectomy compared to the reflux of bile and pancreatic juice. Pancreatic juice reflux appears to exert a co-carcinogenic effect when combined with bile.

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