Original Article

Establishment of animal model of gastroesophageal reflux disease by per-oral endoscopic tunneling: a preliminary study

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Background: Although a variety of antireflux procedures and medications are used to treat gastroesophageal reflux disease (GERD), reliable large-animal models of GERD that can be used to objectively compare the efficacy of these treatments are lacking. We developed a method to establish large animal models of GERD by endoscopic sphincterotomy to develop an endoscopic treatment for GERD.

Methods: In this study six flesh swine carcasses were used. A full thickness incision was made at the esophageal site 5 cm above the dentate line by per-oral endoscopic tunneling. Esophageal radiography was conducted before and after surgery to observe changes at the site of the lower esophagus 5 cm above the dentate line and in the cardia.

Results: There was no significant change in the diameter of the esophageal site 5 cm above the dentate line before and after surgery, while the cardiac orifice significantly relaxed after surgery and enabled the contrast agent to smoothly travel through. The difference in diameter was statistically significant ($P<0.05$).

Conclusions: Our experiments showed that it is a minimally invasive and mature technology of establishing GERD animal models by using the per-oral endoscopic tunneling technique, and might be a new method to establishing GERD large animal models.

Keywords: Gastroesophageal reflux disease (GERD); animal model; swine

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Introduction

Gastroesophageal reflux disease (GERD) refers to the symptoms and complications induced by the reflux of contents of the stomach into the esophagus, oral cavity (including the throat), or lung. GERD is a common disorder of upper gastrointestinal motility, which seriously threatens human health, especially in Western developed countries (1). The mechanism of this disease is the dysfunction of the lower esophageal sphincter (LES) induced by the decrease of the anti-reflux mechanism, the enhancement of damage of reflux content to the esophageal mucosa, and the decrease in esophageal acid clearance. In addition, the high pressure region of the gastroesophageal junction is considered to be the key factor to prevent reflux. Continuous reflux of the stomach and duodenal fluid has great influence on the daily life of an individual. Studies have revealed that approximately 10% of these patients develop reflux esophagitis, Barrett's esophagus and lower esophageal adenocarcinoma (2-5). Proton-pump inhibitor therapy is the main treatment method for GERD, which

can relieve symptoms in most patients, and show a very good curative effect. However, this treatment only controls its symptoms instead of curing it, and its curative effect on refractory GERD is poor (6). Anti-reflux surgery is one of the best options for severe GERD patients undergoing long-term treatment. At present, endoscopic treatment cannot replace traditional medicine or surgical treatment due to efficacy and safety problems. Therefore, a simple, safe and effective GERD therapy is needed. We developed a method to establish large animal models of GERD using the endoscopic technique. Animal models provide a comprehensive platform for the study of the function and anatomy of the esophagus and stomach, and provides better basis for future studies of the endoscopic treatment of GERD.

Methods

Experimental materials

Olympus GIF-Q260 host (Olympus Corporation, Japan), Olympus GIT-Q260J gastroscope and graphic system (Olympus Corporation, Japan), Olympus UES-30 high frequency electric transmitter (Olympus Corporation, Japan), C-arm X-ray machine (GE, USA), camera (Canon, Japan), mucosal needle (Medwork, USA), and Olympus heat hemostatic forceps and triangular knives (Olympus, Japan).

Experimental animals

In this study, six fresh swine carcasses were used (male or female). The body weight of these carcasses ranged between 35.0–40.0 kg, with an average of 37.14±2.86 kg. No upper gastrointestinal endoscopy training was carried out, which is in accordance to the regulations of the animal experiment.

Experimental animals and processing

Six fresh swine carcasses were used. Before surgery, a self-made endoscopic pad (a 50 mL syringe was used, the piston was pulled, and the end that was connected to the needle was cut off) was placed, and was fixed in the oral cavity of the carcasses using medical tape (*Figure 1*).

Surgical procedures

(I) The swine was placed in the supine position. The upper part of the body was raised by 30 degrees and fixed on the contrast bed; (II) esophageal angiography was carried out with 20% meglumine through the gastroscopic channel, and the diameters of the esophagus 5 cm above the dentate line and cardiac orifice were measured; (III) the swine was placed in the left lying position and fixed on the operation table; (IV) a submucosal injection of methylene blue saline solution (1:10,000) was given at the site 10 cm above the dentate line and the site was lifted; (V) an inverted T-shaped incision of 2 cm in length was performed on the mucosa using a triangular knife, the endoscope was inserted through the incision into the submucosal space, and a tunnel to the site 3 cm under the cardia was established; (VI) with the help of a triangular knife, the whole layer of the muscularis propria was cut down from 8 cm above the cardia to 3 cm under the cardia (*Figure 2*); (VII) the swine was fixed on the contrast bed in the supine position with the upper part raised by 30 degrees, and the diameter of the esophagus 5 cm above the dentate line and cardiac orifice was measured again.

Statistical analysis

Experimental data were analyzed using SPSS 17.0 statistical software. The diameters in these two groups were expressed as mean ± standard deviation (SD). Data obtained at the same site were compared using independent sample *t*-test. $P<0.05$ was considered statistically significant.

Results

Comparison of esophageal and cardiac diameters before and after surgery (*Tables 1*,*2*): a full thickness incision was made on the muscularis propria at the site 5 cm above the dentate line after tunneling. Before and after surgery the diameters of the esophagus 5 cm above the dentate line and cardiac orifice were measured by X-ray radiography and compared (*Figure 3*). The diameters of esophagus and cardia were 13.167±2.787 and 1.167±0.683 at 5 cm on the preoperative dentate line, and 14.833±3.764 and 4.833±0.753 respectively postoperative. There was no significant change in esophageal diameter at 5 cm on the dentate line before and after operation. $P>0.05$, was not statistically significant. Cardiac mouth was significantly relaxed after surgery, contrast agent through the smooth. The difference in diameter was statistically significant ($P<0.05$).

Discussion

GERD is a common disease of the digestive system that has various symptoms, extremely easily relapses, and is

Figure 1 Fixed the simple of swine carcass, esophageal angiography was carried out through the gastroscopic channel. (A) A self-made endoscopic oral pad was placed and fixed with adhesive tape in the oral cavity of the swine; (B) the upper part of the body was raised by 30 degrees and fixed on the contrast bed; (C) esophageal angiography was conducted through the endoscopic biopsy channel; (D) the swine was placed in the left lying position and fixed on the operating bed.

a refractory chronic disease (7,8). Current treatments include medical, surgical and interventional treatments; but clinical efficacy remains limited (9-11). In order to more scientifically study the mechanism of the occurrence and development of GERD, or to develop new treatment methods, more and more scholars have been involved in related animal experiments. Since the cause and mechanism of this disease remains unknown, it has become a difficulty in related scientific experiments to establish a reasonable and accepted animal model. These methods can be classified into two categories: esophagitis induced by reflux caused by changes in the gastrointestinal structure, and esophagitis induced by exogenous acid filling (12). With the continuous development of digestive endoscopic technology, the application range of digestive endoscopic treatment has also become more extensive. In 2007, the first description of a peroral endoscopic myotomy (POEM) on an animal model was done by a group of North Americans for achalasia (13). In 2009, En-Qiang Linghu, a professor, presented a special report on "*Endoscopic submucosal tunnel dissection (ESTD) treatment of lesions in esophageal ring*" at the Beijing Digestive Endoscopy Conference, which was named endoscopic tunneling technology (14). In 2010, Inoue, a Japanese scholar (15), reported the technology, POEM. In recent years, the application of POEM treatment for achalasia has annually increased due to its relative satisfactory efficacy and overall safety (16). In follow-ups after POEM, mild reflux symptoms occurred in few patients. In this study, we

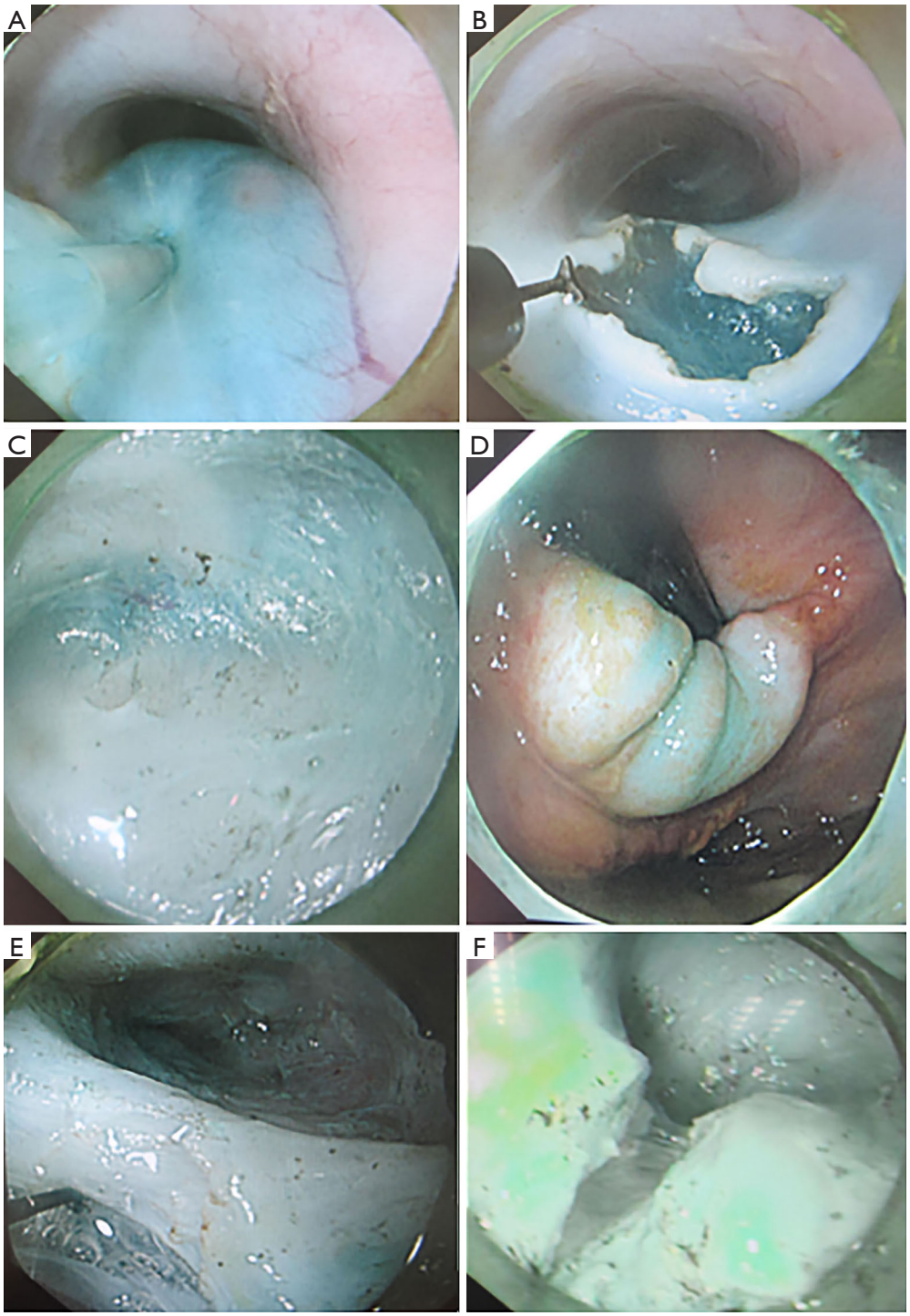

Figure 2 Build the submucosal tunnel to 3 cm under the cardia. (A) Submucosal injection was conducted at 10 cm above the dentate line with the aid of transoral endoscopy; (B) incision of the mucosa was performed using a triangular knife; (C) a submucosal tunnel was established; (D) a tunnel under the cardia was established; (E,F) full thickness incision of the muscularis propria from 8 cm above the cardia to under the cardia was performed.

were inspired from these reports, and proposed whether the tunnel technology can be used to establish a large animal model of gastroesophageal reflux.

In general, animal models help more widely and objectively evaluate various therapeutic methods compared with clinical studies. In the 1970s, Bremner *et al.* (17) carried out gastroesophagectomy through the removal of LES, in order to establish the first GERD animal model. Then, the animal model established by cardiomyotomy was extensive applied. In recent years, through constant experimental studies, according to the anatomical characteristics of different animals, McMahon *et al.* (18), Gawad *et al.* (19) and Poorkhalkali *et al.* (20) respectively carried out surgical incisions of the LES of dogs, swines and cats. Hence, effective and reliable GERD animal models were established.

A number of animal models have been attempted in the past, but they did not have endoscopic tunneling technology. In the present study, the model of gastroesophageal reflux was established by endoscopically establishing a submucosal tunnel to the cardia to damage the LES. The LES is a physiological sphincter at the junction of the esophagus and stomach. This area is a relatively high pressure area that can prevent the reflux of the stomach and duodenal contents into the esophagus. The gastroesophageal junction of a swine is very similar to

Table 1 Esophageal and cardiac diameter before and after surgery

Sample	Preoperative		Postoperative	
	Esophagus (mm)	Cardia (mm)	Esophagus (mm)	Cardia (mm)
1	12	2	12	4
2	17	1	20	5
3	15	1	17	5
4	12	2	13	6
5	9	2	10	4
6	14	1	17	5

Table 2 Esophageal and cardia diameter before and after surgery test results comparison

Group	Preoperative	Postoperative					
	Mean ± SD (mm)	Mean ± SD (mm)	T value	P value	Difference mean	Standard error	95% CI
Esophagus	13.167±2.787	14.833±3.764	0.872	0.4038	1.667	1.912	–2.59 to 5.93
Cardia	1.167±0.683	4.833±0.753	8.771	0.0000	3.333	0.380	2.49 to 4.18

CI, confidence interval; SD, standard deviation.

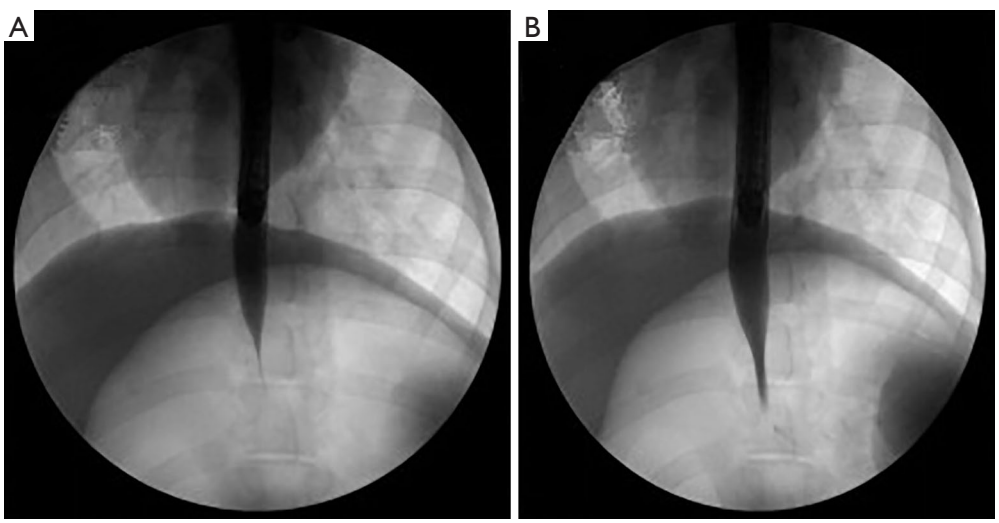

Figure 3 Esophageal angiography was conducted again, and results were compared with angiography results post-surgery. (A) Pre-surgery angiography results; (B) post-surgery angiography results.

that of humans. The surgical incision of LES reduces the pressure of the lower esophageal high pressure zone and damages its normal physiological function, thereby leading to gastroesophageal reflux. Angiography before surgery revealed that the gastroesophageal junction is tightly closed. Furthermore, after incision of the lower esophagus and sphincter, radiography revealed that gastroesophageal junction was obvious relaxed; and enabled the contrast agent to smoothly travel through. We will also conduct further studies on living swines, measure the pressure of the gastroesophageal junction, and perform 24-hour PH monitoring, in order to more objectively evaluate the establishment of the gastroesophageal animal model. Through our previous animal experiments, a large animal model of gastroesophageal reflux was established using the endoscopic tunneling technology, providing better basis for future studies of GERD.

Conclusions

Our animal experiments showed that it is a minimally invasive and mature technology of establishing GERD animal models by using the per-oral endoscopic tunneling technique, and might be a new method to establishing GERD large animal models, and can provide animal models which are more close to human anatomy for GERD basis studies and treatment.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: Principles of Laboratory Animal Care' (NIH Publication Vol 25, No. 28 revised 1996) were followed, as well as specific national laws (e.g., the current version of the German Law on the Protection of Animals) where applicable. The animal study protocol had been approved by the Medical Ethics Committee of Inner Mongolia Medical University. The animal experiments were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals'.

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