Comparison of two porcine benign esophageal stricture models using radiofrequency ablation and endoscopic submucosal tunnel dissection

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ABSTRACT

Background/Aims: *Large-animal benign esophageal stricture (BES) models are needed for the development of new endoscopic therapies and related devices. This study was undertaken to develop and compare swine BES models produced by radiofrequency ablation (RFA) or endoscopic submucosal tunnel dissection (ESTD).*

Materials and Methods: *RFA and ESTD were each performed on three pigs. Follow-up endoscopy and esophagography were performed immediately after the procedures and then 2, 3, and 4 weeks later. Four weeks after the procedures, all animals were sacrificed, and gross and histologic examinations were performed.*

Results: *BES was successfully achieved in both the RFA and ESTD groups, and all animals survived without any serious adverse events during the 4-week follow-up period. Mean procedural times were 9.3 min for RFA and 89.3 min for ESTD. ESTD caused long segment strictures whose average length was 4.5 cm, whereas RFA produced short strictures whose average length was 1.4 cm. BES began to form 2 weeks after both procedures. Degrees of strictures were similar at 3 and 4 weeks in the ESTD group; however, it started deteriorating over time in the RFA group. Histologic examinations showed that ESTD caused inflammation and fibrosis in the submucosal layer, whereas RFA induced extensive inflammation in the submucosal and muscularis propria layers.*

Conclusion: *BES was successfully achieved using RFA or ESTD in swine without serious complications. The methods have different characteristics; therefore, researchers should choose the method more appropriate for their purposes.*

Keywords: *Stricture, esophageal, submucosal dissections, endoscopic, ablation, radiofrequency catheter*

INTRODUCTION

Benign esophageal stricture (BES) is not a rare disease in clinical practice and can occur after endoscopic resection, esophageal surgery, corrosive injury, or radiotherapy (1). Most cases can be endoscopically managed, such as by balloon dilation, bougie, or stenting, but some are refractory and require frequent endoscopic procedures, which introduce the risk of perforation. Therefore, a large-animal BES model is required to aid the development of new endoscopic treatments and related devices.

Various methods, such as photodynamic therapy (PDT), corrosive injury, and endoscopic mucosal resection (EMR), have been used to induce BES in small- and large-animal models (2-8). PDT is a Food and Drug Administration-approved treatment for esophageal cancer. The technique is straightforward and reproducible, and PDT doses can be varied to control the degree of stenosis in given animals. On the contrary, it requires the use of an expensive photosensitizer and laser equipment (3). NaOH induces corrosive injury of the esophagus leading to BES, and the induction of BES using NaOH is straightforward and does not require complicated, expensive equipment. However, the doses required to induce BES have not been established, and NaOH can cause serious adverse events due to esophageal burns or perforation and sometimes even death (2,6,8). EMR and endoscopic submucosal dissection (ESD) have been widely used to produce large-animal models of BES, but conventional EMR and ESD require skilled endoscopists, are time-consuming, and have high procedure-related complication rates (e.g., bleeding and perforation) (7,9).

No optimal method has been devised to create a large-animal BES model; therefore, simple, safe, and economical

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methods are needed. The aims of the current study were to develop large-animal models of BES using radiofrequency ablation (RFA) or endoscopic submucosal tunnel dissection (ESTD) and to compare the effectiveness, safety, and reproducibility of these two models.

MATERIALS AND METHODS

Animals

Six female mini pigs (*Susscrofa*, Cronex Co., Ltd, Hwaseong, South Korea) with weights ranging between 35 and 40 kg were used in this study. The six pigs were equally assigned to the RFA or ESTD groups. After a 24-h fast, animals were anesthetized using an intramuscular injection of tiletamine/zolazepam and xylazine. After endotracheal intubation, general anesthesia was maintained during endoscopic procedures by administering 1.5%-2.0% isoflurane in oxygen inhalation. Pigs were allowed a soft diet on the day following the procedure. The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the sponsoring institution (KNOTUS IACUC: 16-KE-287).

Radiofrequency ablation

A 7F biliary RFA catheter with a working length of 175 cm (ELRA™ RF catheter, STARmed, Goyang, Korea) was used. This catheter was originally designed for clinical applications on bile ducts. It was a disposable bipolar device and suitable for the endoluminal delivery of RF into tubular structures. Energy was delivered using an RFA generator (VCS10™, STARmed) in a temperature-controlled mode.

An expert endoscopist (S.J.) familiar with endobiliary RFA performed the endoscopic RFA procedure. After placing a transparent cap-fitted gastroscope (GIF 260J™, Olympus, Tokyo, Japan) in the distal esophagus (50 cm from upper incisors), an RFA catheter was introduced through its working channel, which allowed the RFA electrode to move beyond the distal end of the endoscope. First, the RFA electrode was placed at the four o'clock position of esophageal mucosa; subsequently, the RFA electrode was positioned in close contact with the esophageal wall by sucking air from esophageal lumen. Esophageal RFA was performed under suction. A 22-mm-long RFA electrode was used for the experiment using the following conditions: 10 W, a target temperature of 80°C, and an ablation time of 120 s. The energy settings and ablation time used were determined by referring to a previously described RFA-induced biliary stricture model (10). After endoscopic examination of the first esophageal RFA site, RFA was repeated at the same level at the 10 o'clock position in the esophagus in the same manner (Figure 1).

ESTD

Upper endoscopy was performed by another expert endoscopist (B.W.B.) using a standard 9.8-mm gastroscope (GIF 260J™, Olympus). A transparent cap was attached to the tip of the endoscope to observe the submucosal layer in detail. In the same way as ESD, an electrosurgical unit (VIO 300D, ERBE, Tübingen, Germany) was connected to provide cutting or coagulation when needed. Esophageal ESTD was performed as shown in Figure 2. After injection of submucosal saline solution, circumferential incision was performed using a Dual Knife™ (Olympus) at 10 cm above the esophagogastric junction. Submucosal saline solution was injected at 5 cm above the circumferential incision and a submucosal tunnel was subsequently created which extended to the circumferential incision. On the opposite side of the submucosal tunnel, another submucosal tunnel was created in the same way. After completing the two tunnels, dissection was performed using a Dual Knife on the border between the submucosal tunnels to completely remove circumferential esophageal

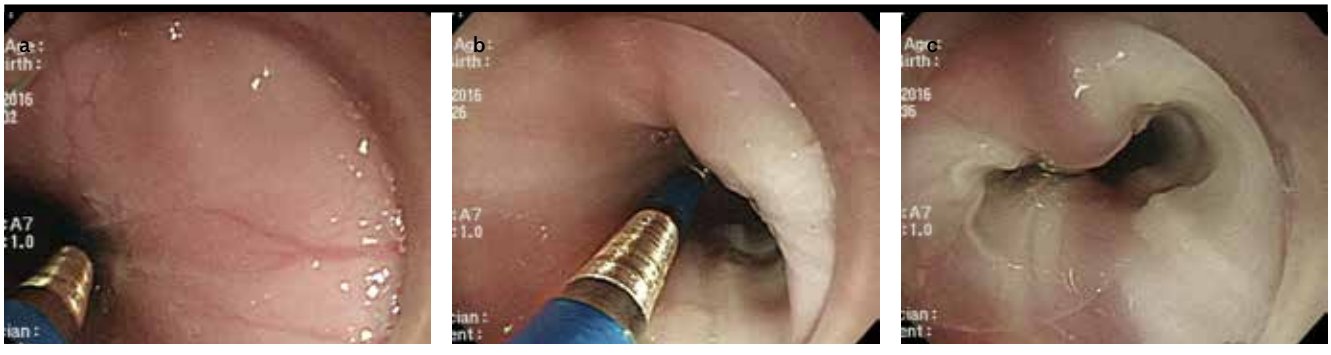

Figure 1. RFA method: After the RFA catheter contacted the esophageal wall, RFA was performed while the RFA catheter was attached to the esophageal wall by suction (a); after RFA had been performed at the 4 o'clock position, esophageal mucosa turned white and esophageal stricture was induced (b); after the first RFA application, second RFA was performed at the 10 o'clock position (c)

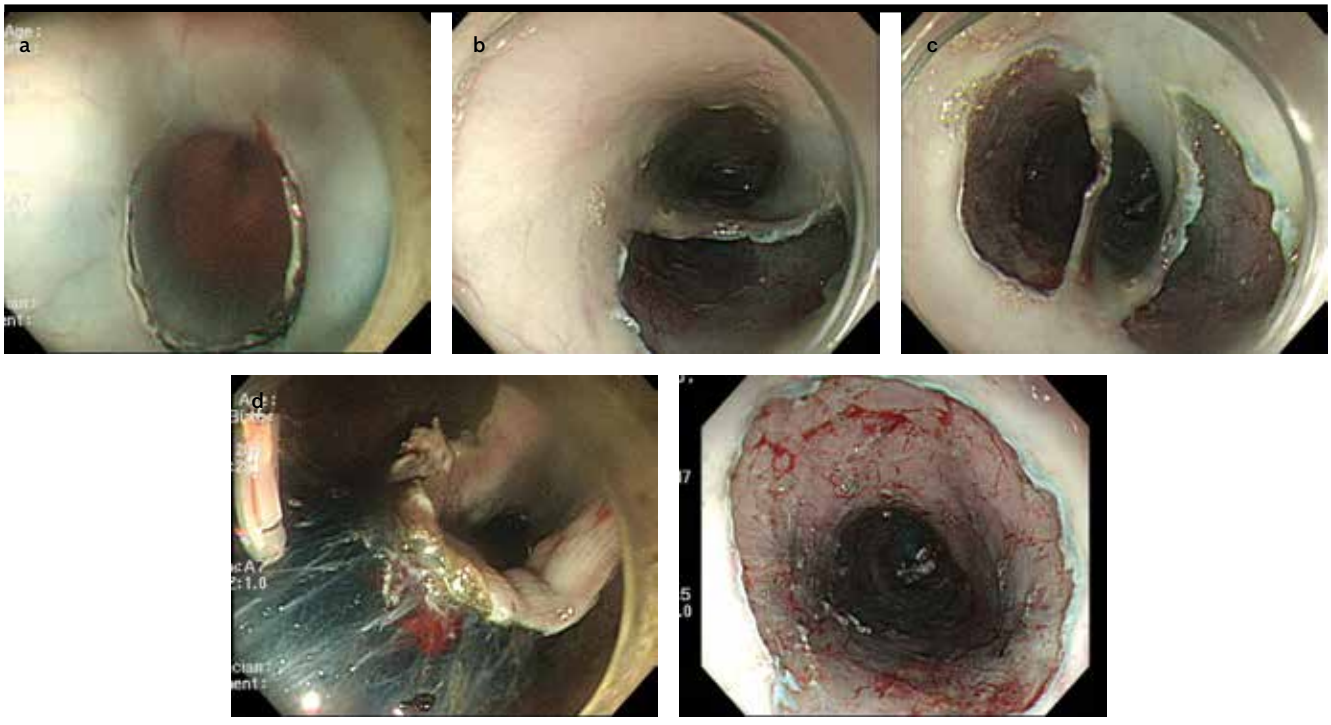

Figure 2. ESTD method: After submucosal saline solution injection, circumferential incision was performed at 10 cm above the gastroesophageal junction (a); two submucosal tunnels were created on the opposite sides of the esophageal wall 5 cm above the circumferential incision (b, c); the border of the submucosal tunnel was dissected after the submucosal saline solution injection (d); completion of the double-tunnel ESTD (e)

mucosa. A hemostatic forcep (Coagrasper™, FD-410LR; Olympus) was then applied to coagulate visible vessels and to prevent delayed bleeding.

Post-procedure follow-up

During the 4 weeks following both procedures, animals were fed a liquid diet, and clinical signs and parameters, including body weight and food intake, were monitored daily. Follow-up endoscopy and esophagography were performed immediately after procedures and then 2, 3, and 4 weeks later. Stricture length was calculated using endoscope diameter (9.8 mm) in esophagograms as a reference.

Data collection

Procedure times were measured from endoscope insertion to procedure completion. Investigators prospectively collected clinical data, such as, vital sign, body weight, and food intake, on a weekly basis.

Histologic examination

Esophagi were extracted at 4 weeks after procedures and evaluated grossly and under a light microscope. For histologic study, specimens were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin and Masson's trichrome.

Table 1. Clinical outcomes of the experimental animals

No.	Method	Procedure time (min)	Stricture length	body weight change (%)	Complication
1	RFA	11	1.6	-11.8	None
2	RFA	9	1.0	5.0	None
3	RFA	8	1.5	-6.6	Fistula formation
4	ESTD	98	4.1	0.2	None
5	ESTD	90	4.9	-5.8	None
6	ESTD	80	4.6	-5.0	None

RFA: radiofrequency ablation; ESTD: endoscopic submucosal tunnel dissection

RESULTS

Animals

The six animals survived the designated 4-week follow-up period, and no serious adverse event, such as perforation

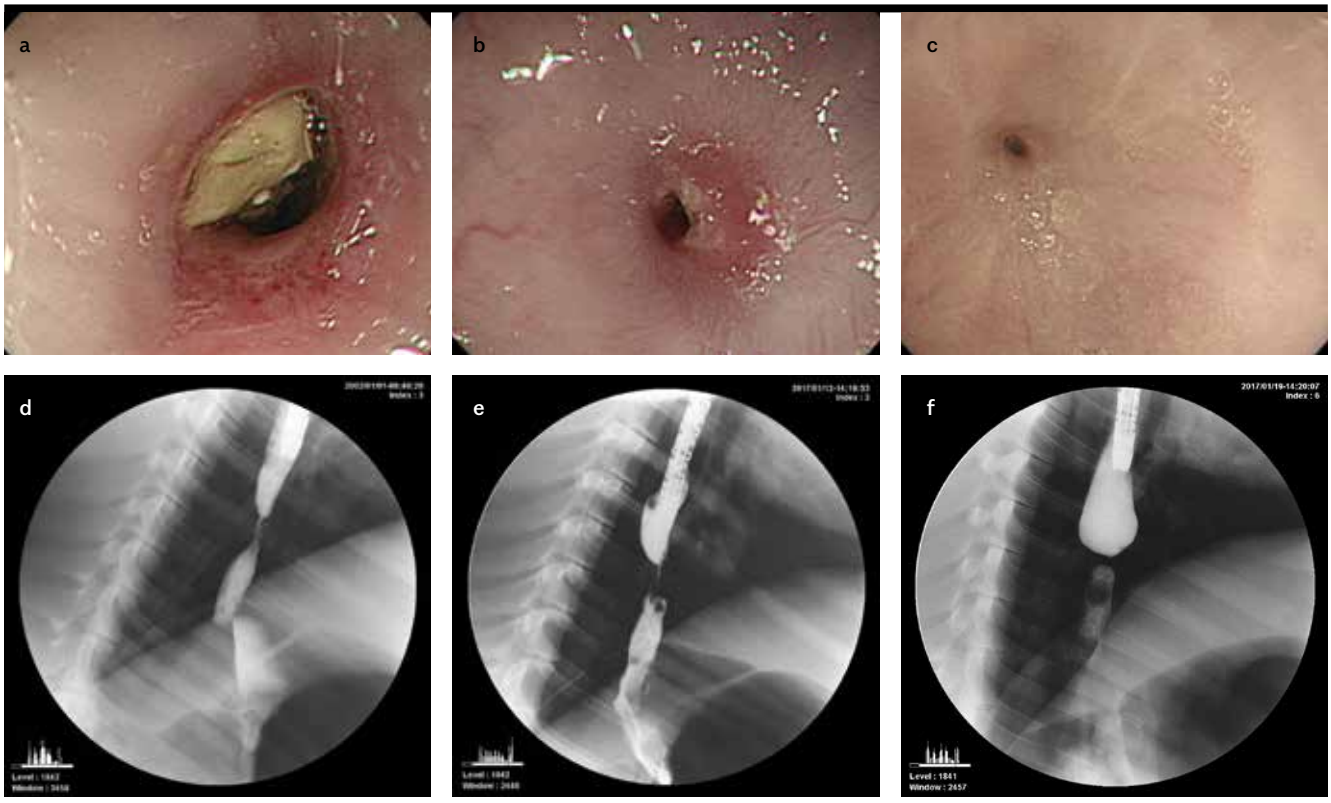

Figure 3. RFA: At 2 weeks after RFA, endoscopy revealed esophageal stricture with necrotic tissue (a); stricture degree worsened between weeks 3 and 4 after the procedure (b, c); esophagography findings also showed that strictures worsened over time, and at 4 weeks after RFA, flow of contrast dye through strictures was severely restricted (d-f)

or massive bleeding, was encountered. No abnormal clinical findings were observed during follow-up, and only one animal in the RFA group exhibited more than 10% weight loss at 4 weeks after the procedure (Table 1).

Outcome measurement of the procedures

RFA and ESTD procedures were successfully performed in all animals. No technical difficulties or procedure-related adverse events were encountered during these procedures. ESTD produced long segment strictures whose average length was 4.5 cm, whereas RFA caused short segment strictures whose average length was 1.4 cm. Mean procedure times for RFA and ESTD were 9.3 and 89.3 min, respectively (Table 1).

Serial endoscopies and esophagograms

At 2 weeks post-RFA, esophageal stricture with central ulcer and necrotic tissue attributed to thermal injury were endoscopically observed, and stricture severity was found to worsen between weeks 3 and 4. Esophagography also showed that strictures deteriorated with time, and contrast dye flow through stricture sites was limited at 4 weeks (Figure 3). At 4 weeks, one animal was found to have an esophageal fistula. In the ESTD group, esophageal stricture developed at 2 weeks and was slightly aggravated at 3 weeks after ESTD, but no further aggravation occurred between 3 and 4 weeks (Figure 4).

Histologic findings

In the RFA group, although the mucosal layer was preserved, severe and extensive inflammation and fibrosis were observed in the submucosal and muscularis propria layers. In the ESTD group, the mucosal layer was absent and severe inflammation and fibrosis were observed in the submucosal layer. However, unlike that observed in the RFA group, the muscularis propria layer was preserved in the ESTD group (Figure 5).

DISCUSSION

In this study, we successfully developed BES in swine using RFA- and ESTD-based methods. Strictures developed 2 weeks after both procedures, and significant strictures were formed at 3 weeks post-procedurally. Although only 3 animals were allocated to each method, both methods were found to be effective and safe because all animals

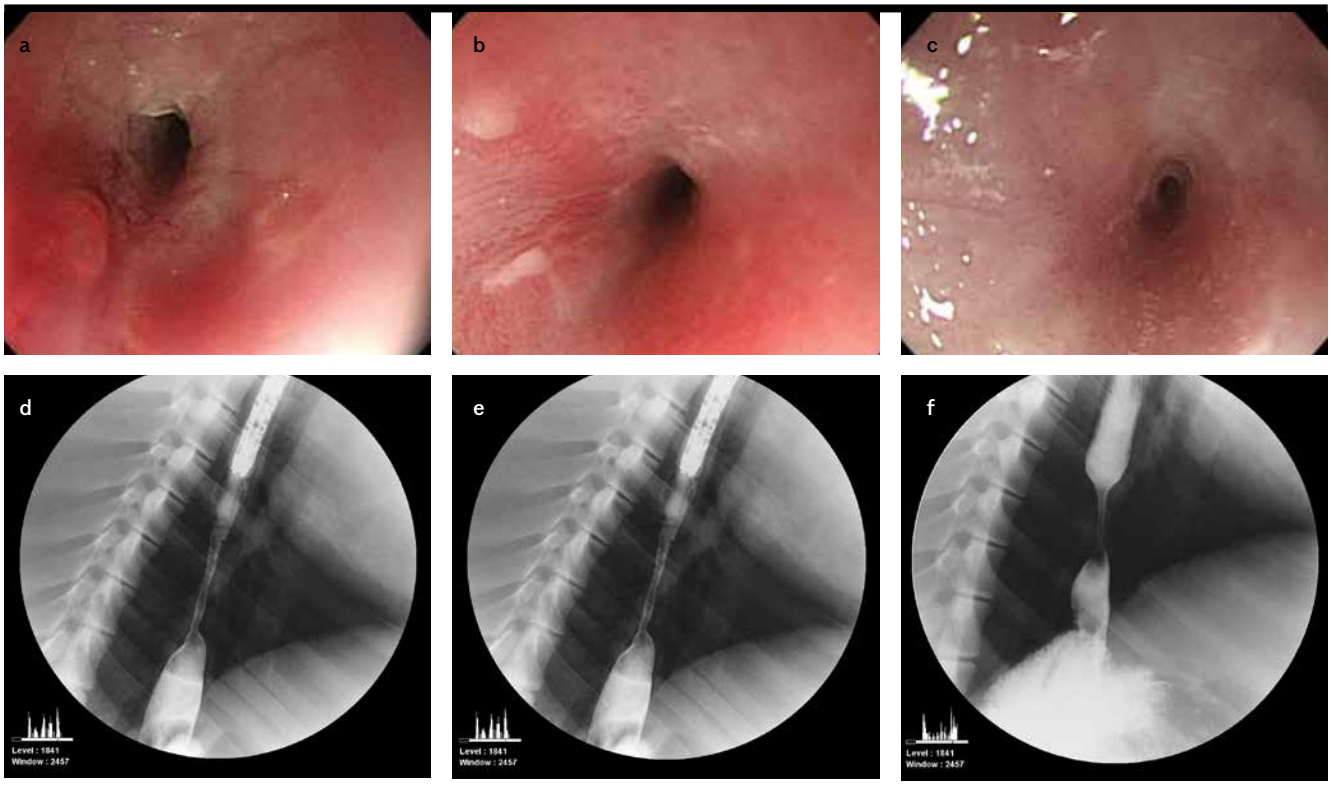

Figure 4. ESTD: Endoscopic findings showed esophageal stricture development at 2 weeks after ESTD (a); and slight stricture aggravation at 3 weeks after ESTD (b); but no interval change between 3 and 4 weeks after ESTD (c); esophagography depicted long segment strictures, but no significant change in stricture severity over time (d-f)

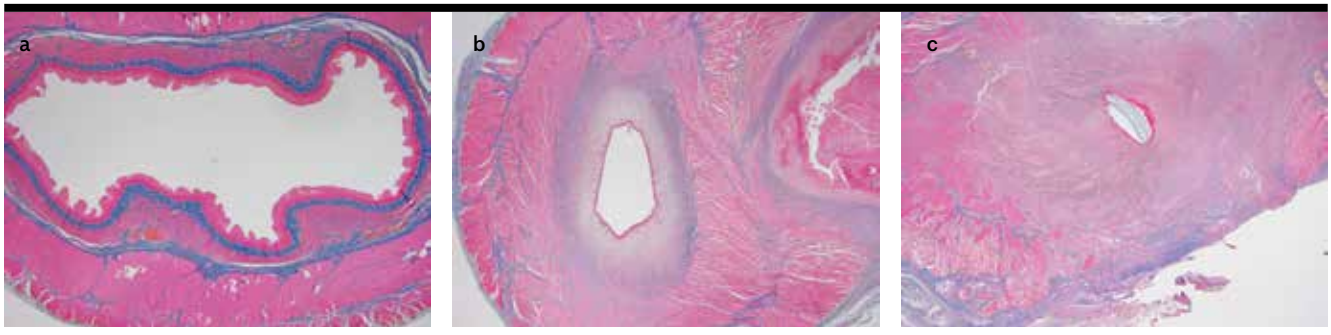

Figure 5. Histologic findings of esophagi (Masson's trichrome stain, 12.5×): Normal esophagus shows a preserved esophageal layer (a); after ESTD shows the absence of a mucosa layer and severe fibrosis and inflammation in the submucosal layer. However, the muscularis propria layer was preserved (b); after RFA shows the mucosal layer was preserved, but inflammatory cells and fibrosis were observed extensively throughout the submucosal and muscularis propria layers (c)

developed significant stricture and survived the experiments. However, the two developed methods have their advantages and disadvantages.

RFA has been used effectively and safely in several gastrointestinal disorders (11-13). RFA delivers heat to tissue, which causes necrosis around the RFA catheter and ultimately results in stricture. RFA has several advantages because it is not technically difficult to manipulate and is easily controlled by changing the amount of thermal energy administered (11). Other advantages of RFA include short procedure times and high reproducibility. However, it is difficult to control stricture length, and because RFA electrode lengths are limited, RFA creates relatively short strictures. In addition,

because RFA-induced strictures gradually progress to total occlusion, it is difficult to maintain animal life over the long-term without intervention, such as dilation.

ESTD was originally developed to treat achalasia or subepithelial tumors of the esophagus (14,15). However, the indications for ESTD have extended over recent years to the treatment of large superficial neoplasms of the esophagus (16). ESTD has the advantages of relatively rapid and safe removal of large esophageal lesions as compared with conventional ESD. ESTD enables BESs to be produced in a highly reproducible manner and allows researchers to control the severities and lengths of esophageal strictures, for example, if a mild esophageal stricture is required, 75% circumferential resection could be performed, and if strictures of certain lengths are required, the procedure can be easily adjusted. However, ESTD is disadvantaged by long procedure times, procedure-related complications, and the need for a skilled endoscopist familiar with ESD experience. Stricture development after ESTD occurs because absence of the barrier function of epithelium leads to infections and inflammatory reactions, which result in scarring and stricture (7).

Currently, the developments of endoscopic accessories and stents are hampered by the lack of a suitable large-animal esophageal stricture model. Newly developed esophageal stents are generally investigated for physical properties *in vitro*, and then directly introduced to clinical practice because no reliable large-animal model of esophageal stricture has been devised. Furthermore, *in vivo* animal studies of stent durability and membrane changes are currently being performed by surgically fixing stents to normal esophageal walls (17).

In terms of clinical applications, the RFA-induced BES animal model induces short segment stricture and would thus benefit studies on certain types of esophageal strictures, such as anastomosis site strictures and esophageal obstruction by cancer. On the contrary, the ESTD-induced BES animal model may be suitable for studying corrosive esophageal injury by lye or acid because it can be used to produce long segment strictures.

This study has several limitations that warrant consideration. First, the number of animals included was too small to allow generalizations of experimental results. Second, we used an RFA catheter that was developed for bile duct application. If a balloon-type RFA catheter had been used, it would have been easier to perform the procedure and would have allowed even energy delivery to the esophageal wall, which would have reduced the possibilities of perforation and fistula. Unfortunately, balloon-type RFA catheters are not yet available in Korea.

In conclusion, we successfully developed a large-animal BES model using RFA- or ESTD-based methods, which produced models with different characteristics. Therefore, we recommend researchers choose a method appropriate for intended study by carefully considering the merits and demerits of the devised RFA and ESTD large-animal models of BES.

Ethics Committee Approval: The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the sponsoring institution (KNOTUS IACUC: 16-KE-287).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.J.; Design - D.H.L.; Supervision - D.H.L.; Resources - S.J.; Data Collection and/or Processing - S.J., B.W.B.; Analysis and/or Interpretation - B.W.B.; Writing Manuscript - S.J., B.W.B.; Critical Review - S.J., D.H.L.

Conflict of Interest: The authors have no conflict of interest to declare.

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