Sodium hydroxide-induced esophageal stricture via an endoscopic injection needle: a novel rabbit model of corrosive injury
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ABSTRACT
Purpose: Benign strictures of the esophagus are commonly encountered in clinical practice and are difficult to manage conservatively. This study aimed to establish a novel animal model of benign esophageal stricture by using corrosive-induced injury in rabbits with an injection of sodium hydroxide (NaOH) via a self-made endoscopic injection needle.

Materials and Methods: Corrosive injury of the esophagus was induced in 10 rabbits by administration of 1 mL of 1.5% NaOH using a laryngoscope with a self-made endoscopic injection needle. The self-made injection needle was fabricated by modification of the core of an endoscopic injection needle. The laryngoscope examination was performed at 2 weeks and 4 weeks after induction of corrosive injury; esophagography was also performed at 4 weeks to assess esophageal stricture. All animals were euthanized at the end of the fourth week; the esophagus was removed, and stained sections were examined microscopically.

Results: Laryngoscope examination at 2 weeks showed ulceration. At the end of fourth week, laryngoscopy, radiological, and gross examinations showed successful induction of esophageal stricture in all animals, without any complication. The mean stricture index at the end of fourth week was 49.54±3.61%; the mean length of stricture was 18.0±2.5mm. Microscopic examination revealed focal ulceration and submucosal thickening secondary to fibrosis.

Conclusion: Rabbit esophageal stricture induced using laryngoscopy with endoscopic injection of a small amount of low-concentration sodium hydroxide is a technically simple, safe, and reproducible method for creation of an animal model of esophageal stricture. This model can be useful for developing new treatment methods for esophageal stricture.

Key Words: animal; laryngoscope; self-made endoscopic injection needle; esophageal stenosis; models

INTRODUCTION
Benign esophageal stricture (BES) is a commonly encountered problem in clinical practice, and commonly the result of caustic ingestion, radiotherapy, surgery, or peptic ulceration (1-3). BES can be treated efficiently by serial endoscopic balloon dilation and/or stent insertion (4-6). However, about 10% of the cases are refractory to these measures (5, 6), and management of such cases can be challenging (6, 7). Currently, various procedures are available for treating these refractory cases; however, none of the methods is entirely satisfactory. Thus, an experimental animal model is essential for developing the effective treatment methods and testing the clinical application of new techniques. The present study aimed to establish a new animal model of benign esophageal stricture by laryngoscopy and injection of sodium hydroxide using a self-made endoscopic injection needle.

MATERIALS AND METHODS
Animals
The study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Shanghai Jiao Tong University of Medicine (approval No. amc-40). Ten New Zealand white rabbits (male; 10-week-old; 3.8-4.8 kg) were obtained from the Shanghai Jiao Tong University Animal Center. The animals were maintained at the animal center of the Shanghai Jiao Tong University of Medicine in individual stainless-steel cages and provided standard pellet diet and water ad libitum.

Self-made Laryngoscope Needle
A sterilized endoscopic injection needle was used
to fashion the laryngoscope needle. The shell of the endoscopic injection needle was removed, retaining the inner core with a diameter of approximately 18 mm, which was compatible with the 2-mm working channel of the laryngoscope. About 70 cm of the inner core was harvested (the length of the laryngoscope was 60 cm). The needle tip was shortened to 2 mm. The injection hose was connected to the other end, and a bolus of saline was injected to check the patency. Finally, this self-made needle was sterilized using 75% alcohol (Fig. 1).

**Endoscopic Procedure**

After a 24-h fast, the rabbits were anesthetized with pentobarbital (5 mg/kg) administered via the ear edge vein (8) and positioned in the left lateral decubitus position.

The equipment used included a laryngoscope (GIF-XP260N; Olympus Optical Co., Ltd, Tokyo, Japan), with 60 cm length, 6 mm diameter, and a 2 mm working channel; a 5 Fr balloon catheter with a 12-mm diameter balloon (MTW Endoskopie, Wesel, Germany); a 5 Fr polytetrafluoroethylene (PTFE) tube (Daikin Industries, Ltd, Osaka, Japan). The laryngoscope, equipped with the 5 Fr PTFE tube, was inserted to the mid-esophagus. The balloon catheter was advanced into the esophagus; the balloon inflated in order to prevent a distal leakage of the caustic agent. The self-made needle was introduced through the PTFE tube, and 0.25 mL of 1.5% sodium hydroxide (NaOH) was injected at the four sites around the circumference of the esophagus. The balloon was maintained in an inflated state for 30 s, and then deflated and removed. The antibiotics were not administered since severe enterocolitis could occur in rabbits receiving antibiotics (9).

**Laryngoscopy and Esophagography**

All rabbits were fed normally after the procedure. The esophagus was evaluated by endoscopy 2 weeks after the procedure. The esophageal stricture was assessed by endoscopy and esophagography at 4 weeks. The degree of the stricture was measured during esophagography, with correction for magnification performed using the external diameter of the endoscope’s distal end as the reference. Both the lumen diameter and the length of the stenosis were measured, and the stricture index (lumen diameter at 4 weeks/lumen diameter at 0 week) was calculated. In this study, the esophageal stricture was defined as a stricture index ≤50.

**Histological Examination**

All rabbits were euthanized at the end of the fourth week, and the esophagus was extirpated for gross observation and histological examination. The excised esophagus were fixed in 10% formaldehyde for 48 h, followed by paraffin -embedding. 4 mm transverse sections were obtained at the level of the lesion and stained with hematoxylin and eosin (H&E). Masson’s trichrome staining was also employed to evaluate the expression of collagen.

**RESULTS**

All rabbits survived the procedure. At the end of designated 4-week follow-up period, the animals were weighed before being euthanized. A decrease in body weight (ranging from 40-180 g) was seen in 8 rabbits at the end of fourth week. 2 rabbits showed no change in weight. An endoscopic evaluation at 2 weeks revealed ulceration in all animals. At the end of fourth week, scar formation and stricture were observed in all animals.

**Laryngoscopy Findings**

A follow-up laryngoscopy revealed esophageal mucosal edema and ulcers at 2 weeks (Fig. 2A) and esophageal stricture at 4 weeks (Fig. 2B). After corrosion, the most severe inflammation in the esophageal wall was observed at the end of the second week, and the repair was completed at the end of the fourth week.

**Radiological Findings**

Barium esophagography demonstrated stricture formation in all animals at 4 weeks (Fig. 3). The mean stricture index at the end of the fourth week was 49.54 ± 3.61% (range, 44.89-56.65%) (Table 1).

**Pathological Findings**

Gross examination of the extirpated esophagus revealed esophageal stricture (Table 1) in all animals. The mean length of the stricture was 18.0±2.5 (range, 14.6-22.8) mm. Microscopic examination revealed focal ulceration and submucosal thickening secondary to fibrosis in all specimens (Fig. 4).
Table 1. Changes in esophageal stenosis in models.

| Case | Body weight (kg) | Lumen diameter (mm) | Stricture index (%) | Stricture length (mm) |
|------|------------------|---------------------|---------------------|---------------------|
|      | 0 week | 4 weeks | 0 week | 4 weeks | Specimen | Specimen |
| 1    | 3.05   | 2.98    | 9.7    | 4.5    | 46.39    | 15.9    | 14.5 |
| 2    | 4.00   | 3.82    | 10.4   | 5.5    | 52.88    | 14.6    | 13.1 |
| 3    | 3.26   | 3.18    | 9.8    | 4.4    | 44.89    | 19.2    | 17.8 |
| 4    | 3.60   | 3.60    | 10.3   | 5.5    | 56.65    | 21.4    | 19.0 |
| 5    | 3.85   | 3.81    | 10.3   | 5.4    | 52.43    | 17.6    | 15.7 |
| 6    | 3.20   | 3.16    | 9.9    | 4.7    | 47.47    | 22.8    | 21.1 |
| 7    | 3.44   | 3.44    | 10.0   | 5.1    | 51.00    | 18.9    | 17.3 |
| 8    | 3.92   | 3.85    | 10.1   | 5.1    | 50.49    | 16.7    | 15.0 |
| 9    | 3.68   | 3.60    | 9.8    | 4.5    | 45.45    | 17.5    | 16.1 |
| 10   | 3.50   | 3.45    | 9.7    | 4.6    | 47.41    | 15.4    | 13.8 |
| Mean | 3.56±0.30 | 3.23±0.29 | 11.26±0.25 | 5.58±0.42 | 49.54±3.61 | 18.0±2.5 | 16.3±2.4 |

0 week: preoperative; 4 weeks: postoperative 4 weeks. Stricture index = lumen diameter at 4 weeks / lumen diameter at 0 week.

DISCUSSION

In this study, the novel model of BES was successfully established in all rabbits. To our knowledge, this is the first study using a laryngoscope and a balloon catheter for establishing a simple and reproducible animal model of BES. Previously, the animal models of BES have been successfully established using methods such as corrosive injury, endoscopic mucosal resection (EMR), and photodynamic therapy (PDT) (10-15). Some groups induced the corrosive esophageal injury in rats by surgical isolation of an esophageal segment and instillation of a corrosive agent into that segment (12, 14, 16). Perry et al. (11) induced the esophageal stricture using upper endoscopy and PDT in a pig model. EMR has been used for creating canine models of esophageal stricture (13, 15).

Herein, we selected rabbits for the establishment of the model because of several advantages. First, the rabbit esophagus comprises of three skeletal muscle layers: inner and outer longitudinal layers and a middle circular layer (10). This anatomical arrangement offers resistance to corrosive perforation and allows animal survival until the development of the esophageal stricture. Second, the rabbit is sufficiently a large-sized animal to undergo multiple endoscopic examinations with an ultraslim upper endoscope. Third, the rabbit is easier to handle with cost-effective maintenance than larger animals such as pigs and dogs. Finally, this animal model, due to convenient handling, was developed for exploring the application of esophageal stents.

The method of esophageal stenosis by corrosion is widely used; thus, we selected NaOH to establish the models. The use of NaOH for inducing corrosive injury can lead to infection, respiratory complications directly related to the esophageal burn, and perforation (14). Thompson et al. (10) injected 1.0 mL of 4% NaOH through a 10-Fr plastic catheter for creating esophageal stricture in rabbits. In the study, 4/10 animals died prematurely: 3 due to respiratory complications and 1 due to perforated abdominal viscus. In the current study, we used a lower concentration of NaOH, administering 1.0 mL of 1.5% NaOH through a PTFE tube, with a balloon catheter to avoid any leakage of the NaOH into the stomach. PTFE is a synthetic fluoropolymer of tetrafluoroethylene that possesses inert properties and is commonly known by the brand name Teflon™ (17). Turkyilmaz et al. (12) described an experimental model of caustic esophageal burns in rats. The method described that 2 cm distal of the abdominal esophagus was isolated and tied with 2-0 chronic.
sutures distally and proximally. A 24-Fr cannula was then passed into the isolated segment for instillation of 10% NaOH solution. In the current study, we introduced an ultraslim endoscope equipped with a 5-Fr PTFE tube up to the mid-esophagus to avoid NaOH aspiration into the airway; in addition, a balloon catheter was used to ensure an isolated exposure to NaOH solution and prevent caustic agent leakage into the stomach. Thus, this method allowed the induction of esophageal stricture with a small amount of relatively low-concentration NaOH, with no complications in any of the animals.

The most commonly used methods for creating the corrosive esophagitis model are traumatic, resulting in high complications and mortality rates. Thus, we selected a minimally invasive approach. With the use of the electronic laryngoscope, we could ensure an accurate injection of the NaOH at the selected sites. This method was simple, convenient, and highly reproducible. Pathological examination showed that this model was equivalent to the conventional models such as those created by Gehanno (14) and Senturk (16). Moreover, due to the formation of a submucous fibrous scar, the model was well-suited for investigating the use of stents in esophageal stricture.

Nevertheless, the current method has some advantages over other methods. The use of the PTFE tube and the balloon circumvents the airway and stomach injury due to corrosive leakage. Furthermore, the injection of small amounts of the low-concentration corrosive agent at the 4 sites under direct vision regulates the stricture length and, in addition, dramatically decreases the risk of perforation. Thus, our method is minimally invasive, simple, and reproducible; the rabbit is relatively easy to handle, cost-effective, and optimal for research on stents.

The present study did not compare the innovative model with the conventional corrosive injury animal model. The future studies will compare the two models for data analysis of parameters, such as modeling time.

In conclusion, we have successfully established a novel rabbit model of esophageal stricture by an endoscopic delivery of a small amount of a low-concentration corrosive agent. This model is technically simple, safe, and reproducible and can be used to develop and test new methods of esophageal stricture management.

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