ORIGINAL PAPERS

Adv Clin Exp Med 2015, **24**, 4, 637–641
DOI: 10.17219/acem/22364

ISSN 1899–5276

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Experimental Esophagitis Model Preventing Tracheal Aspiration

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Corrosive esophagitis injuries are a serious clinical problem with many agents used for diagnosis and treatment. Experimental esophagus burn models use a method described mainly in studies by Gehanno and Guedon, and modified by Liu and Richardson.
Objectives. The aim of this study was to describe a new esophagitis model that prevents tracheal aspiration.
Material and Methods. In this study we used 16 Wistar albino rats weighing between 220–240 g. The experimental animals were randomly divided into two groups: the model group (group M, n: 8) and control group (group C, n: 8). The necessary anesthesia was administered. Passing through a median laparotomy incision, the abdomen was entered and in group M and C the esophagus was freed and held by a suture at the gastroesophageal junction. After this procedure, about 1 cm proximal to the gastroesophageal junction, the esophagus was suspended by a suture. The esophagus segment between the two sutures was exposed to 0.1 mL 10% NaOH in group M and 0.1 mL saline in group C for 20 s. Ten days later all experimental animals were sacrificed and their esophagus removed. After dying with hematoxylin and eosine trichrome, the histopathological evaluation results for the rats in all groups were investigated with a light microscope.
Results. Histopathological examination indicated submucosal collagen increase, damage to muscularis mucosa and tunica muscularis and collagen deposition. In the model group, the rats had high neutrophils and tissue damage accompanied by necrosis. In the control group, the rats had minimal or no tissue damage and fibrosis was not observed.
Conclusions. Our procedure is relatively less invasive and easy to apply with corrosive esophagitis only in the required region, and at the same time treatment medications can be easily administered (**Adv Clin Exp Med 2015, 24, 4, 637–641**).

Key words: experimental model, corrosive esophagitis, NaOH, tracheal aspiration.

Corrosive material burns in the upper gastrointestinal system, especially in the esophagus, can cause serious morbidity and mortality ranging from stenosis to perforation. Mucosal burns and perforation, and sometimes mortality, can be observed due to caustic damage in the acute period [1, 2]. Chronic complications may include the development of stricture and esophageal carcinoma. Accidental swallowing of caustic material constitutes a serious medical problem, especially in the childhood period. Of these cases, about 20% develop stricture over time [3, 4].

Studies to develop experimental esophagus burn models have used an invasive model, described by Gehanno and Guedon [5] and modified by Liu and Richardson [6]. Apart from these,

a non-invasive model developed by Erturk et al. has been shown [7].

In this study we aimed to describe a new esophagitis model that prevents tracheal aspiration.

Material and Methods

This study was completed in the Canakkale Onsekiz Mart University experimental animal laboratory after all the required permissions were granted by the Canakkale Onsekiz Mart University Animal Experimentation Ethics Committee chairmanship. For the experiments, 16 Wistar albino rats weighing between 220–240 g were used. The experimental animals were randomly divided into 2 groups; the model group (group M, n: 8) and the control group (group C, n: 8). Both groups of rats exposed to caustic burns were starved for 12 h before surgery. For surgical intervention in the rats, 50 mg/kg ketamine intraperitoneal (*i.p.*) and 5 mg/kg xylazine *i.p.* were administered for anesthesia. The abdomen was entered through a median laparotomy incision and in group M and C the esophagus was freed (Fig. 1) and suspended from the gastroesophageal junction using 3.0 silk suture (Fig. 2). Later, about 1 cm proximal to the gastroesophageal junction, the esophagus was suspended by 3.0 silk suture (Fig. 3) and the esophagus segment between the two sutures was exposed to 0.1 mL 10% NaOH using a 27 gauge insulin needle for 20 s in group M (Fig. 4 and 5). The rats in group C were exposed to 0.1 mL saline for 20 s. Before the next stage, the distal suture was removed and after 20 s the burned esophagus segment was washed with 10 mL saline. Then the proximal suture was removed, the abdomen was closed and the procedure ended. All experimental animals were free to obtain rat food and water as desired after the operation. Ten days later, all experimental animals were sacrificed under general anesthetic, the esophagus was removed and placed in 10% buffered formalin. Later, after routine histopathological examination, it was covered in paraffin and dyed with hematoxylin and eosine trichrome for

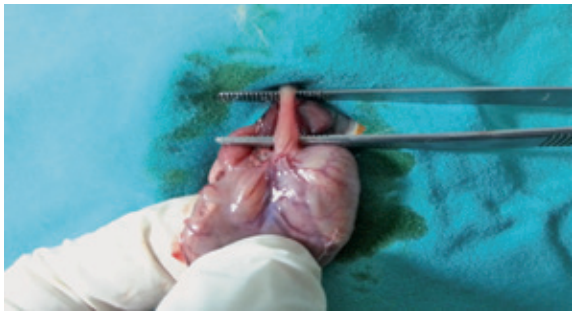

Fig. 1. Dissected esophagus

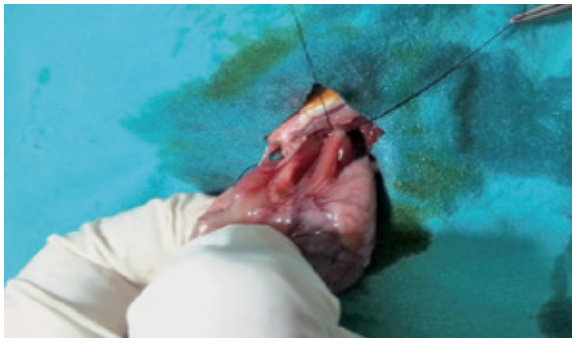

Fig. 2. Gastroesophageal junction has been suspended with suture

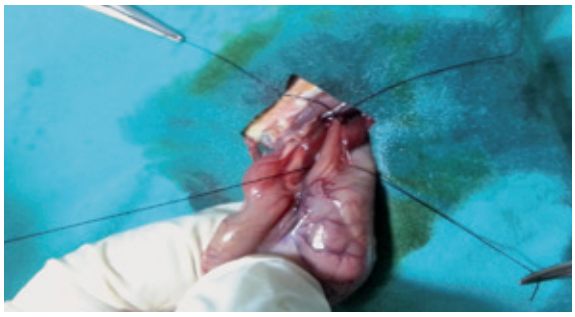

Fig. 3. Esophagus has been suspended with suture from about 1 cm proximal of the gastroesophageal junction

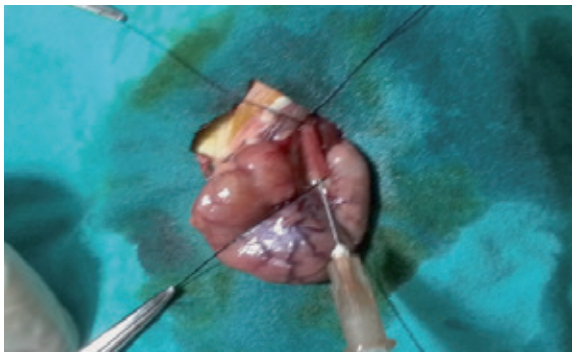

Fig. 4. Esophagus segment between two suspensions was subjected to 0.1 mL of 10% NaOH with a 27 G needle in group M rats

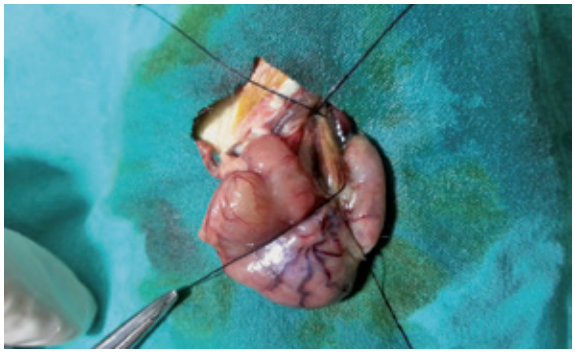

Fig. 5. The view of esophagus segment after the procedure

examination. Four μm slices were made and these sections were examined with a light microscope. Histopathological examination for submucosal collagen increase, muscularis mucosa damage, tunica muscularis damage and collagen deposition was performed for all animals.

SPSS for Microsoft Windows (v. 15.0) was used for statistical analysis. Mann-Whitney *U* or χ^2 tests were used to define the statistical differences between the histopathological parameters. Probability values less than 0.05 were accepted as statistically significant.

Results

The esophagi of the rats sacrificed on the 10th day of the study were examined histopathologically and the results are shown in Table 1. There was inflammation in 2 rats in group C. When compared to group M, the difference was significant ($p < 0.05$). Collagen increase in the submucosa was detected in one rat in group C and collagen was increased in the submucosa of 6 rats in group M. This difference was statistically significant ($p < 0.05$). Injury in the muscularis mucosa was detected in 2 rats in group C and in 5 rats in group M. In the model group rats, neutrophils and necrosis accompanied by tissue damage were present (Fig. 6). In the control group rats, tissue damage was minimal or not present and fibrosis was not observed (Fig. 7).

Discussion

In our method, designed according to the histopathological results in this study, significant differences were observed in the model group in terms of the presence of inflammation, submucosal collagen increase, muscularis mucosa damage, *tunica muscularis* damage and collagen deposition compared to the control group.

Caustic agents have great capacity to cause damage to mucosal surfaces. Minor damage developing after caustic burns is characterized by simple erythema and edema of mucosal surfaces. However, it may cause deep tissue necrosis progressing to catastrophic perforation leading to mortality.

Table 1. Histopathological results of experimental groups

	Group C (n = 8)	Group M (n = 8)	P
Inflammation	2+/6–	7+/1–	< 0.005
Collagen increase in submucosa	1+/7–	6+/2–	< 0.005
Damage to the muscularis mucosa	2+/6–	5+/3–	< 0.005
Damage and collagen deposition in the tunica muscularis	1+/7–	5+/3–	< 0.005

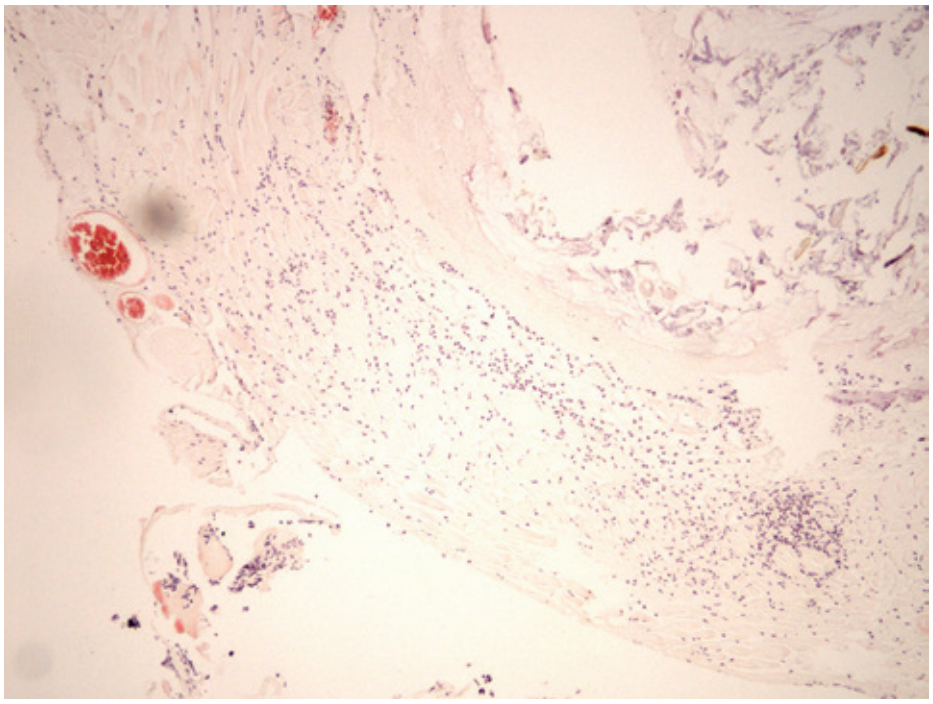

Fig. 6. Severe inflammation with polymorphonuclear infiltration, more severe inflammation with ulcers in model groups (H&E ×100)

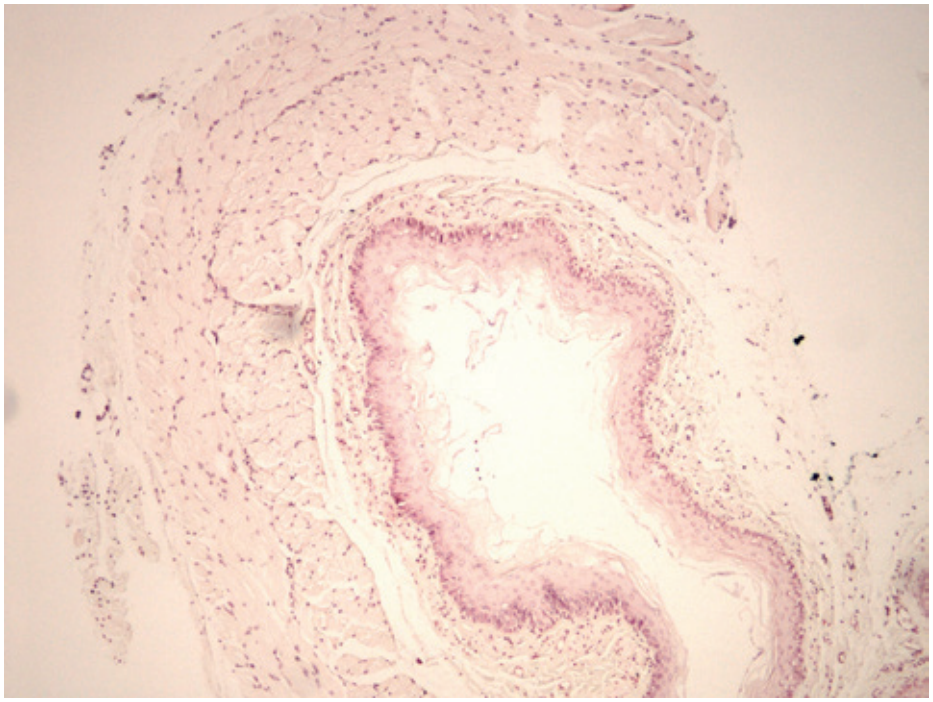

Fig. 7. Minimal inflammatory changes in control groups (H&E ×100)

Burns due to alkali agents, especially, may cause deep penetration and liquefaction necrosis of esophageal tissue [8–10]. In our study, to evaluate the effectiveness of our model on histopathological examination, characteristics such as inflammatory cell infiltration, muscularis mucosa damage and collagen deposition were researched. All rats in the model group were observed to have esophageal burns while, different to the control group, severe inflammatory cell infiltration was observed

The most commonly used invasive model for corrosive esophagitis is the model of Gehanno et al. [5] However different modifications of this model may be used [6]. In this model, subjects are starved for 12 h before the procedure and laparotomy uses a midline incision under general anesthetic. After laparotomy, the stomach is found and brought outside the incision and the cardia and a 1.5 cm segment of the abdominal esophagus is suspended with silk suture material. Afterwards, advancing along the oroesophageal path, a nasogastric catheter is placed to reach the center of the suspended esophagus segment. Nearly 1.5 cm above the cardia, proximal to this segment, the esophagus is tied with suture so reflux will not occur. Afterwards, the front portion of the stomach and 0.5 cm away from the cardia is punctured and a cannula is located in the distal esophagus lumen. Thus an isolated segment is formed within the distal esophagus lumen with two catheters and NaOH is administered to this isolated segment. Later the esophagus is freed, the stomach is closed and the laparotomy is ended [5]. The difference with our model is that a nasogastric probe is not placed and an additional cannulation from the stomach to the esophagus is not completed. This completely isolates the esophagus segment to create the burn and removes the risk of tracheal aspiration and pharyngeal burns.

Before creating the esophagus burn in esophagitis, models by Erturk et al [7]. used an endoscope to measure the esophagus length of the rats and to create the burn using a Fogarty catheter within a guide. The balloon of the Fogarty catheter is expanded to close the entrance to the stomach. While they used 40% NaOH to create esophagus burns, our model used 10% NaOH. In addition, this procedure is extremely laborious and at any stage has a risk of esophagus perforation, stomach perforation and, though low, tracheal aspiration.

As described in the literature, it is technically not possible to administer 1–1.5 mL of chemical to a 1–1.5 cm segment of the esophagus [11]. The total volume of the esophagus in 300 g Wistar rats is about 0.3 mL [11, 12]. The amount of chemical agent should not be more than 0.3 mL for the lower part of the esophagus [13]. In our model, 0.1 mL 10% NaOH was used to create the burn and was observed to be sufficient. Our model does not have a risk of tracheal aspiration, as other models do. In addition, in the literature, while there are many cases of pharyngeal burns, in our study no rat was observed to have tracheal aspiration or pharyngeal burns.

This experimental research should be accepted as a starting point for studies examining broader experimental animal series and changes on longer timescales. In addition, the limitations of our study include that it is only histopathological, the rats were sacrificed after a short time so it does not

evaluate esophagus stricture that may develop in the long term, and there is no sham group.

In conclusion, research and discussion on the treatment of corrosive burns of the esophagus should continue. We hope the model we applied provides a positive contribution to these studies. As planned in our model, corrosive esophagitis was observed in only the distal part of the esophagus. In other models, more than the end part of the distal esophagus, burns are created in the whole esophagus. Our model is relatively less invasive and easily applied, providing corrosive esophagitis in the desired region and at the same time allows easy administration of treatment medications.

References

[1] **Larios-Arceo F, Ortiz GG, Huerta M, Leal-Cortés C, Saldaña JA, Bitzer-Quintero OK, Rodríguez-Reynoso S:** Protective effects of melatonin against caustic esophageal burn injury in rats. J Pineal Res 2008, 45, 219–223.

[2] **De Jong AL, Macdonald R, Ein S, Forte V, Turner A:** Corrosive esophagitis in children: a 30-year review. Int J Pediatr Otorhinolaryngol 2001, 57, 203–211.

[3] **Karnak I, Tanyel FC, Büyükpamukçu N, Hiçsönmez A:** Combined use of steroid, antibiotics and early bougienage against stricture formation following caustic esophageal burns. J Cardiovasc Surg (Torino) 1999, 40, 307–310.

[4] **Mutaf O:** Treatment of corrosive esophageal strictures by long-term stenting. J Pediatr Surg 1996, 31, 681–685.

[5] **Gehanno P, Guedon C:** Inhibition of experimental lye stricture by penicillamine. Arch Otolaryngol 1981, 107, 145–147.

[6] **Liu AJ, Richardson MA:** Effects of N-acetylcysteine on experimentally induced esophageal lye injury. Ann Rhinol Laryngol 1985, 94, 477–482.

[7] **Senturk E, Sen S, Pabuccu E, Unsal C, Meteoglu I:** New experimental corrosive esophagitis model in rats. Pediatr Surg Int 2010, 26, 257–261.

[8] **Lupa M, Magne J, Guarisco JL, Amedee R:** Update on the diagnosis and treatment of caustic ingestion. Ochsner J 2009, 9, 54–59.

[9] **Özçelik MF, Pekmezci S, Saribeyoğlu K, Unal E, Gümüştaş K, Doğusoy G:** The effect of halofuginone, a specific inhibitor of collagen type 1 synthesis, in the prevention of esophageal strictures related to caustic injury. Am J Surg 2004, 187, 257–260.

[10] **Holinger LD:** Caustic osephagial burn. In: Swenson's Pediatric Surgery. Ed: Raffensperger JG, Apleton and Lange, Connecticut 1990, 5th ed., 827–831.

[11] **Hebel R, Stromberg MW:** Anatomy of the laboratory rat. Williams & Wilkins, Baltimore 1976, 43–52.

[12] **Cochran ST, Fonkalsrud EW, Gyepes MT:** Complete obstruction of the gastric antrum in children following acid ingestion. Arch Surg 1978, 113, 308–312.

[13] **Koltuksuz U, Mutus HM, Kutlu R, Ozyurt H, Cetin S, Karaman A Gürbüz N, Akyol O, Aydin NE:** Effects of caffeic acid phenethyl ester and epidermal growth factor on the development of caustic esophageal stricture in rats. J Pediatr Surg 2001, 36, 1504–1509.

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Conflict of interest: None delcared

Received: 20.01.2014
Revised: 04.03.2014
Accepted: 18.06.2014