Title page

Title: Investigation of surgical technique for bronchial stump closure after lobectomy in animal model

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Abstract

Objective: Bronchial fistulae following lung surgery are associated with high mortality. We examined the histological effects of mucosal ablation as a technique for closing bronchial stumps to prevent bronchial fistulae in an animal model.

Methods: Left lower lobectomy was performed in beagles under general anesthesia. The bronchial stumps were closed using one of the following four methods: (A) manual suturing using 3-0 absorbable sutures, (B) ablation of bronchial mucosa with electric cautery and manual sutures, (C) stapling and reinforcement with manual sutures, or (D) ablation and stapling followed by reinforcement with manual sutures. Bronchial stumps were histologically evaluated on postoperative day 14.

Results: No bronchial fistulae were noted in the animals during the observation period. Histologically, there were no adhesions between the bronchial mucosae at the suture and staple lesions in groups A and C. The bronchial mucosae were adherent at the ablation sites in groups B and D. Inflammatory cells, myofibroblasts, and neovascular vessels were abundant around the ablated lesions.

Conclusions: Bronchial mucosal ablation may play a key role in mucosal adhesion and tight union of the bronchial stump.

INTRODUCTION

Bronchial fistula is an established severe complication of lung surgery, with a reported incidence of 1.3%–12%. For lobectomy, the incidence of bronchial fistula is less than 2% [1-7]. However, the occurrence of bronchial fistula is associated with a high rate of subsequent mortality. Therefore, a safe and effective procedure for bronchial closure is required to prevent bronchial fistula. Several surgical techniques for preventing bronchial fistula have been reported, including coverage using different types of autologous tissues [8, 9] and surgical glues [10] that are commonly used to close bronchial stumps.

Rienhoff et al. suggested that wound healing does not primarily occur at the site of sutures on the bronchial stump and that mucosal surfaces cannot completely unite at the site [11]. We hypothesized that primary mucosal tight adhesion could augment bronchial wound healing and that bronchial mucosal ablation could contribute to mucosal tight adhesions. In the present study, we examined the histological effects of mucosal ablation as a surgical technique for closing bronchial stumps in order to prevent bronchial fistulae in an experimental animal model.

MATERIALS AND METHODS

Animals and surgical procedure

In line with previous research [12], four adult female beagles (Kitayama Labes Co. Ltd., Nagano, Japan) weighing 10 kg each were used in this study. After pre-anesthetization with ketaminol (10 mg/kg) and atropine sulfate (0.25 mg), anesthesia was induced with intravenous injections of propofol (5 mg/kg body weight) and succinylcholine (1 mg/kg body weight) and maintained with periodic injections of propofol (1 mg/kg body weight) and succinylcholine (0.25 mg/kg body weight). The animals were placed in the right lateral position, and a left lower lobectomy was performed via left thoracotomy in each animal. Tracheal intubation was performed using a single endotracheal tube with an internal diameter of 7.5 mm. Intraoperative hydration was maintained with Ringer's solution (10 mL/kg body weight) via the cephalic vein, and respiration was maintained with mechanical ventilation using pressure cycles. Based on the treatment of the bronchial stump, the animals were classified into 4 groups. In group A (n=1), the bronchial stump was sutured manually using 3-0 absorbable sutures. The suturing technique used was single ligation with Sweet's method (Fig. 1a). In group B (n=1), the bronchial mucosa of the stump was ablated using electrocautery and sutured manually, as in group A (Fig. 1b, Fig. 2). In group C (n=1), the bronchial stump was stapled using linear staplers (TA stapler, Covidien, Japan) and reinforced using manual sutures at the distal end, as in group A, without

mucosal ablation (Fig. 1c). In group D (n=1), the bronchial mucosa of the stump was stapled and reinforced using manual sutures at the distal end with mucosal ablation, as in group B (Fig. 1d).

The chest wall was closed after air evacuation via the chest tube. The chest tube was removed before extubation. The animals were returned to their cages after intramuscular injections with ketoprofen (1 mg/kg) and ampicillin sodium (15 mg/kg). The mucosal surface of the bronchial stump was coagulated with a 1-mm wide electrocautery device (ForceTriad™ Energy Platform; Medtronic plc. Dublin, Ireland) using the Monopolar Cut (Blend) mode at 30 W of power for periods of 1-2 seconds.

Histological analysis

Histological analysis was performed 14 days after surgery (n=1 in each group). To evaluate bronchial healing, the beagles were euthanized with an overdose of pentobarbital, and the sites of the bronchial stumps were resected along with the left lungs. The specimens were fixed in formalin and embedded in paraffin. Subsequently, 4 μm thick sections from these samples were analyzed histopathologically using hematoxylin–eosin (HE) and α-smooth muscle actin (α-SMA) staining.

Immunohistochemistry

Immunohistochemical staining for α-SMA was performed using the indirect polymer immunoperoxidase method. Anti α-SMA antibody Clone 1A4 (DAKO, Glostrup, Denmark) was the primary antibody used.

The study protocols were approved by the Ethics Committee of Hiroshima University, and the appropriate national guidelines for the care of laboratory animals were observed.

RESULTS

Macroscopic findings

All animals survived the procedure, and bronchial fistulae were not found in any of the four groups throughout the observation period. No other signs of dehiscence, hematoma, necrosis, or inflammation in the thoracic cavity were observed at the time of euthanasia.

Microscopic findings

Histological examination 2 weeks after the operations revealed a thick epithelium and

infiltration with inflammatory cells in each of the groups.

HE staining: In group A, the bronchial mucosae were not adhered together; however, in group B, with mucosal ablation, they were found to be adhered tightly. In group A, infiltration with very few inflammatory cells was noted (Fig. 3a, e), while dense granulation was observed around the ablation area in group B (Fig. 3b, f). In group C, in which the mucosal end was not ablated, neither adhesion nor inflammation was detected on the stump (Fig. 3c, g). In group D, in which the mucosal end was ablated, bronchial adhesion and inflammation were observed, similar to that in group B, with many inflammatory cells (Fig. 3d, h).

α-SMA staining: There were no cells positive for α-SMA-staining in groups A (Fig. 4a) and C (Fig. 4c). In contrast, there was high expression of α-SMA-stained positive cells, such as neovascular vessels and myofibroblasts, in the granulation tissue in groups B (Fig. 4b, e) and D (Fig. 4d, f), which corresponded to the tight adhesion area.

DISCUSSION

In this study, no bronchial fistulae were noted in any animals during the observation period. Histologically, no adhesions were seen between the bronchial mucosae at the suture and staple lesions in groups A and C, while the bronchial mucosae were adherent at the ablation sites in groups B and D. Inflammatory cells, myofibroblasts, and neovascular vessels were abundant around the ablated lesions.

For a long time, manual suturing techniques, such as the one described by Sweet [13] have been used to treat bronchial stumps. More recently, with advancement in surgical device technology, mechanical auto-suture techniques have been gaining popularity. However, similar to the conventional methods, the auto-suturing techniques cannot always prevent the incidence of bronchial fistulae. Our animal study results showed that bronchial mucosal adhesion was not seen with the conventional Sweet manual or staple suture method. In contrast, when the bronchial mucosae were ablated before implementing the conventional bronchial stump closure technique, complete union of the mucosae was observed. We believe that our technique can help reinforce closure and decrease the incidence of bronchial fistulae.

Generally, the process of wound healing has 3 phases: (1) inflammatory phase, (2) proliferation phase, and (3) remodeling phase [14-17]. The inflammatory phase is marked by aggregation of platelets, coagulation, and infiltration with leukocytes. The proliferation phase is characterized

by re-epithelialization, angiogenesis, fibroplasia, and wound contraction. The inflammatory and proliferative phases were both observed on postoperative day 14, corresponding to the ablated lesion. The inflammatory phase is generally considered to begin soon after injury and is followed by the proliferative phase. The proliferative phase starts within days of the injury [14]. Persistent inflammation, which is an excess immune reaction to mucosal ablation, can last for about 2 weeks, and is likely to cause robust adhesion. Finally, the remodeling phase takes place over a period of months, during which the epithelium responds to the injury by producing collagen and matrix proteins [14-17]. These processes are the result of complex pathways involving regulated reactions. Furthermore, it is well known that some pro-inflammatory cytokines also regulate these reactions.

Because the inflammatory phase is generally ongoing on day 14, and because the proliferative phase begins roughly at this time, we selected day 14 as the date for the histological evaluation. Based on our histological results, HE staining showed infiltration of the ablated areas by inflammatory cells, such as macrophages, neutrophils, and lymphocytes, and adhesions between the bronchial mucosae in groups B and D. These results indicate the formation of granulation tissue and, hence, the inflammatory phase. In contrast, in the groups with non-ablated lesions, fewer inflammatory cells were seen, and the mucosae could easily be separated, regardless of the suturing technique used. In groups A and C, the expression of inflammatory cells was lower

than that at the sites of ablated lesions in groups B and D. These results indicate that no granulation tissue was formed in groups A and C.

We examined the expression of α-SMA (Fig. 4) that mainly represents myofibroblasts, which play an important role in the healing process and regulate the remodeling of connective tissues by combining the extracellular matrix-synthesizing features of fibroblasts through immunohistochemical analysis [12, 18-20]. Theoretically the possibility of impairment of blood supply after mucosal ablation cannot be denied, however α-SMA staining showed abundant neovascularization at the ablated area (Fig. 4e and f).

Our study had some unavoidable limitations. First, our animal model included a limited duration of observation of 2 weeks and a limited number of animals owing to the ethical recommendations for animal protection that suggests sacrificing a minimal number of animals. Second, we examined the stumps histologically, since we thought that accurate evaluation of local inflammation by mucosal ablation was important. As a result, we could not evaluate the functional status, for example pressure resistance test, at bronchial stumps in the same specimen.

CONCLUSIONS

In summary, our methods demonstrates that primary wound healing could involve mucosal tight adhesions after mucosal ablation in animals. It also shows that mucosal ablation could act as a trigger for wound healing and allow primary closure of the bronchial stump. The histological results of this study demonstrated that bronchial mucosal ablation is simple but has potential as a surgical technique for mucosal adhesion.

Acknowledgments

Conflict of interest: The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Figure legends

Figure 1

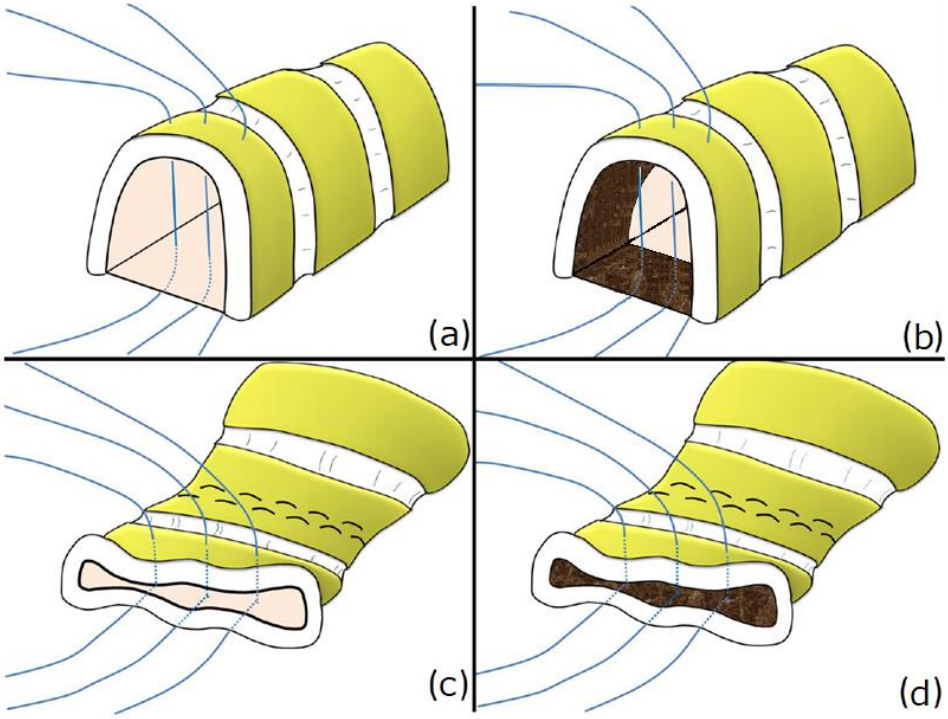

Figure 1: The 4 methods for closing the bronchial stump (a-d). In each group, the suturing technique used was single ligation using the Sweet's method. (a) The bronchial stump was sutured manually using 3-0 absorbable sutures (Group A). (b) The bronchial mucosa was ablated using electrocautery and sutured manually (Group B). (c) The bronchial stump was stapled and reinforced with manual sutures (Group C). (d) The bronchial mucosa was ablated and stapled, and subsequently reinforced with manual sutures (Group D).

Figure 2

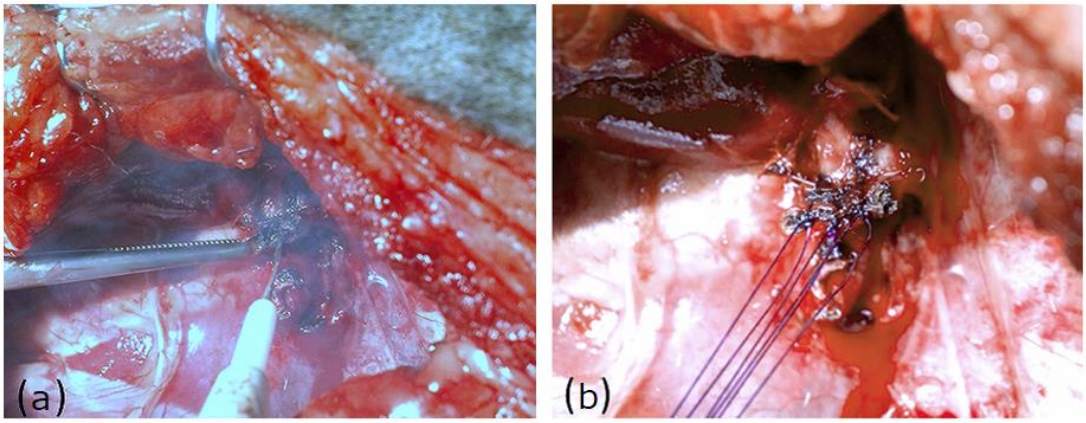

Figure 2: Images showing surgical procedure in an animal model. The bronchial mucosa of the stump was ablated using electrocautery (a) and sutured manually (b).

Figure 3-1

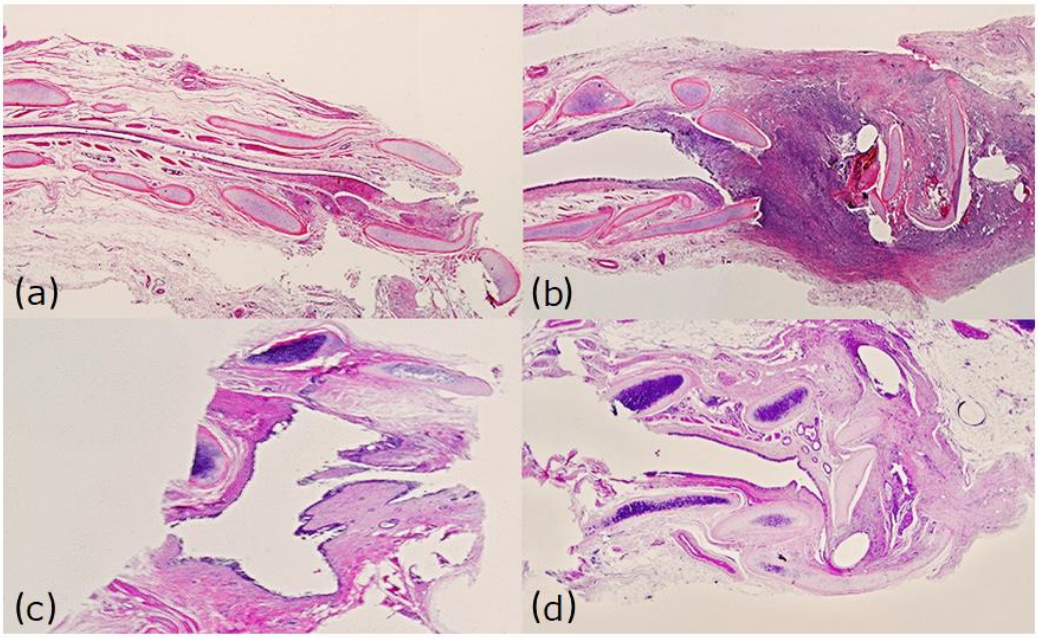

Figure 3-2

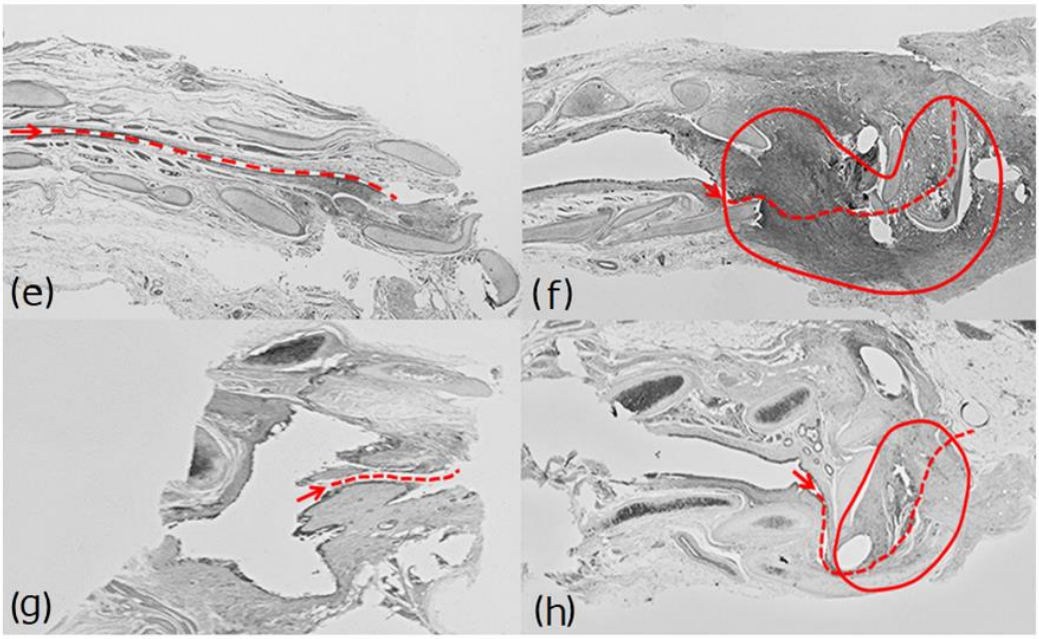

Figure 3: Postoperatively, the bronchial stumps were visualized by hematoxylin–eosin staining. (a-d) at 40× magnification. Grayscale images of each hematoxylin–eosin staining clearly show adhesion from ablation depending on the level of color depth (e-h). The red arrows indicate the innermost layer of the sutured bronchial mucosa, and the dotted red lines indicate the surface of the bronchial mucosa. The red circles indicate the lesion of mucosal tight adhesion.

Figure 4

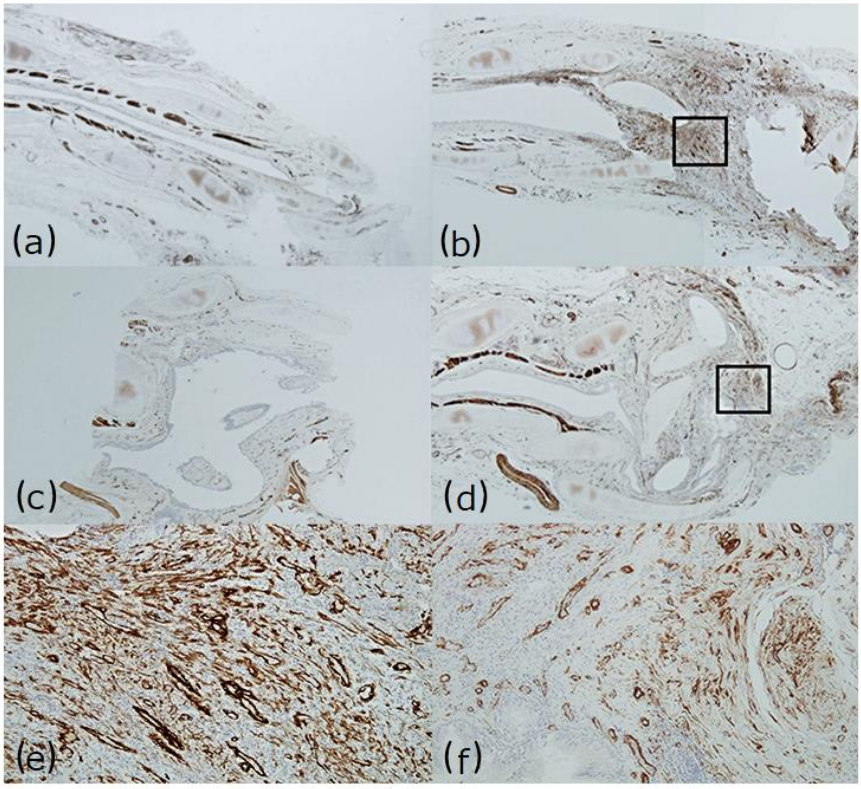

Figure 4: Images showing α-SMA staining (a-d). (a-d) at 40× magnification and (e, f) at 100× magnification. (e) An enlargement of the square area in (b). (f) An enlargement of the square area in (d).

α-SMA, alpha-smooth muscle actin