Bronchoscopic Lung Volume Reduction in a Sheep Model of Heterogeneous Emphysema

Larry Tsai, MD, Andrew Hoffman, DVM, DVSc,† Robert Berger, MD,‡ and Edward Ingenito, MD, PhD**

Abstract: We have previously described the use of bronchoscopic lung volume reduction (BLVR), a novel, tissue engineering-based, minimally invasive technique, in experimental homogeneous pulmonary emphysema in sheep. In this report, we describe the use of CT-guided, site-directed BLVR in the treatment of experimental heterogeneous emphysema. Nine sheep were treated with localized papain instillations and nebulized papain to generate heterogeneous emphysema. Six animals subsequently underwent BLVR while the remaining 3 served as controls. The procedure was safe and well tolerated. BLVR reduced gas trapping (26.7%) as measured by RV-to-TLC ratio, improved diffusing capacity, and reduced a bullous lesion seen by CT scan.

Key Words: Emphysema, Volume reduction, Bullectomy, Bronchoscopy, Tissue engineering

(*J Bronchol* 2004;11:83–86)

Lung volume reduction surgery has recently been shown in a large randomized, controlled trial to be a useful adjunct to medical therapy for patients with advanced pulmonary emphysema, producing substantial improvements in pulmonary function, dyspnea, and quality of life.[1] Nevertheless, perioperative morbidity and mortality remain considerable for this procedure,[2–6] and there has been increasing interest in developing less invasive means of accomplishing similar benefits. Various investigators have explored the use of proximally obstructing mechanical devices, including balloons,[7] "spigots,"[8] and 1-way valves[9–11] designed to reduce volume by limiting ventilation to a portion of lung. These approaches have met with only limited success, most likely due to prominent collateral ventilation in the emphysematous lung, which allows airflow to bypass the device and maintain inflation of the distal lung.[12,13]

We have previously described the development of a novel, tissue engineering-based technique, bronchoscopic lung volume reduction (BLVR), and its successful application in a sheep model of homogeneous emphysema.[14,15] This approach overcomes some of the limitations of proximally obstructing devices by collapsing and scarring an entire subsegment of lung, effectively eliminating both direct and collateral ventilation. To verify further the safety and efficacy of this approach, we applied it in a model of emphysema that more accurately mimics human disease. Emphysema, as it occurs in humans, is generally macroscopically heterogeneous with some severely affected areas and others that are nearly normal. This can have dramatic physiologic consequences and has important implications for volume reduction therapy, because treatment must target only the most diseased areas to be safe and maximally effective. To produce heterogeneous emphysema, we added localized instillations of papain to our emphysema generation protocol.[16] After physiologic and radiographic characterization of the model, we performed BLVR to test its safety and efficacy in heterogeneous emphysema.

MATERIALS AND METHODS

All interventions, CT imaging, and physiologic measurements were performed under light anesthesia (intravenous propofol, 0.5–1.0 mg/kg) while mechanical ventilation was administered through an endotracheal tube.

Emphysema Generation

Nine sheep were treated with bolus papain (1.5 U/kg) delivered through a catheter positioned under bronchoscopic guidance into 6 distal target areas in the lung. Areas were chosen to include both dependent and nondependent zones of cranial and caudal lobes. Each animal also received nebulized papain (75 U/kg) once per week for 3 weeks. The animals were divided into 2 groups: a treatment group that received BLVR therapy (n = 6) and a control group that received sham treatment (n = 3).

Physiologic and Radiographic Measurements

Physiologic and radiographic measurements were made in each animal at 4 time points: prior to papain exposure (base-

From the *Brigham and Women's Hospital, Boston, MA, †Tufts University School of Veterinary Medicine, North Grafton, MA, and ‡Harvard Medical School, Boston, MA.

Supported by NIH grant HL 62266-03; and Bistech, Inc., Woburn, MA.

Larry Tsai owns equity in and receives salary support from Bistech, Inc. Andrew Hoffman owns equity in and serves as a consultant for Bistech, Inc. Robert Berger owns equity in and receives salary support from Bistech, Inc. Edward Ingenito owns equity in and receives salary support from Bistech, Inc.

Reprints: Larry Tsai, MD, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115 (e-mail: ltsai@partners.org).

line [BAS]), after development of emphysema (EMPH), 1 month after BLVR or sham treatment, and 3 months after BLVR or sham treatment. CT scans were obtained at total lung capacity (TLC; 30 cmH_2O distending pressure). Plethysmographic lung volumes were measured by the method of Dubois et al.[17] Pressure–volume relationships (PV) for the lung and chest wall were obtained with an esophageal balloon and supersyringe and were used to construct Campbell diagrams from which residual volume (RV) and TLC were derived.[18] Diffusing capacity was measured with a He/CO analyzer and supersyringe.

BLVR Treatments

BLVR therapy was performed as follows: A flexible endoscope (Olympus Corporation, GIF-N30 pediatric gastroscope, Tokyo, Japan) was introduced through the endotracheal tube and advanced into a subsegmental bronchus. Sites were identified with the assistance of CT scans. The bronchoscope was advanced to wedge position, and 10 mL of Primer Solution (Bistech, Inc., Woburn, MA; containing 0.25% porcine trypsin dissolved in 0.02% Na-EDTA aqueous buffer; pH, 7.6) was injected and left in place for 2 minutes. Suction was then applied for 1 minute to remove as much Primer Solution as possible. Ten milliliters Washout Solution (Bistech, Inc.; containing RPMI 1640 tissue culture media [pH, 7.6] and 10% fetal bovine serum) was then injected and left in place for 1 minute to neutralize any residual Primer Solution. Suction was again applied to remove as much residual solution as possible. A double lumen catheter with separate injection ports for Thrombin and Fibrinogen Solutions was then inserted through the instrument channel and past the tip of the bronchoscope under direct visualization. One milliliter Thrombin Solution (Bistech, Inc.; containing 1000 U/mL bovine serum thrombin in PBS with 5 mM $CaCl_2$) and 10 mL Fibrinogen Solution (Bistech, Inc.; containing 3% bovine serum fibrinogen, 0.1% shark cartilage chondroitin-6-sulfate, 0.1% poly-L-lysine [MW, 70–130,000], and 0.2% tetracycline in RPMI 1640 cell culture media) were injected simultaneously. After 30 seconds, sufficient for complete polymerization, the catheter was removed, the site was inspected, and the procedure was then repeated at the next target site. Treatment at each site required approximately 5 to 7 minutes to complete. Three animals in the treatment group received BLVR at 6 sites, whereas the remaining 3 received BLVR at 10 sites. On completion of BLVR, anesthesia was discontinued and animals were returned to their stalls.

Clinical Evaluation

Peripheral oxygen saturations were measured before, during, and after BLVR. Animals were monitored daily for fever or signs of respiratory distress. Activity level, eating, urination, and defecation were recorded.

Pathologic Evaluation

Animals were euthanized at the 3-month time point. Lungs were removed en bloc and inflated with a supersyringe to look for areas of gross collapse. Samples were collected from collapsed and noncollapsed areas and fixed in 10% buffered formalin prior to sectioning and staining with hematoxylin–eosin (H&E). Samples of heart, liver, kidney, and spleen were also collected.

Statistics

Physiologic data were compared between groups and time points by ANOVA and *t*-test when appropriate. Statistical significance was defined as $P < 0.05$.

RESULTS

Clinical Outcomes

All animals recovered quickly from BLVR and were ambulatory and eating within 1 hour of completion of the procedure. After treatment, 1 of 6 animals experienced a fever (temperature >104°F) that lasted for 4 days, but resolved without specific therapy. None of the animals experienced hypoxia or respiratory distress during the procedure or at any time thereafter.

Physiologic Outcomes

Physiologic measurements demonstrated that after papain treatment, trapped gas, assessed by RV-to-TLC ratio, increased 21.6% (BAS, 0.37 ± 0.15 L vs. EMPH, 0.45 ± 0.09 L; $P = 0.058$). At 1-month follow-up, there was a 16% reduction

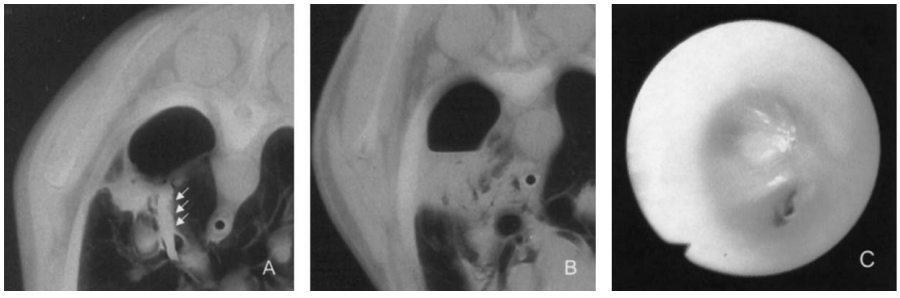

FIGURE 1. A, CT scan showing bronchoscope (arrows) in place in airway proximal to bulla. B, CT scan showing bulla partly filled with BLVR reagents after removal of bronchoscope. C, Bronchoscopic view of polymerized Fibrinogen Solution in subsegmental airway.

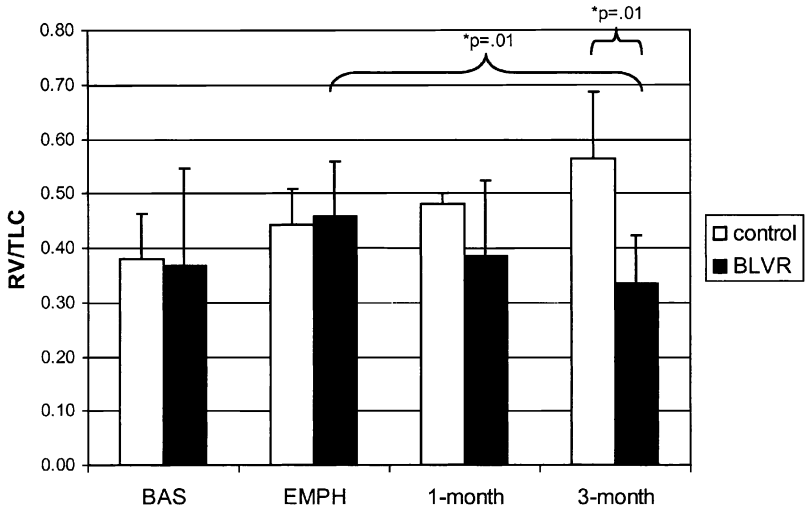

FIGURE 2. RV-to-TLC ratios for control and BLVR-treated animals. Error bars represent 1 standard deviation. Statistically significant changes are marked.

among the treatment animals ($EMPH_{BLVR}$, 0.46 ± 0.10 L vs. 1-$month_{BLVR}$, 0.39 ± 0.14 L, *P* not significant). By contrast, control animals demonstrated further worsening at the 1-month follow-up ($EMPH_{CONTROL}$, 0.44 ± 0.06 vs. 1-$month_{CONTROL}$, 0.48 ± 0.02 L; *P* not significant). At the 3-month follow-up there was a 26.7% improvement in the treatment group, which reached statistical significance ($EMPH_{BLVR}$, 0.46 ± 0.10 L vs. 3-$month_{BLVR}$, 0.33 ± 0.09 L; *P* = 0.01). In contrast, in the control group, progressive gas-trapping occurred with further increases in RV-to-TLC ($EMPH_{CONTROL}$, 0.44 ± 0.06 L vs. 3-$month_{CONTROL}$, 0.56 ± 0.12 L; *P* not significant). Lung volumes are summarized in Figure 2. Diffusing capacities in control and treatment animals displayed a similar pattern. D_1/V_A in control animals remained substantially reduced at 3 months compared with baseline (BAS, 2.48 ± 1.40 mL/min/torr vs. 3-$month_{BLVR}$, 1.55 ± 0.48 mL/min/torr), whereas values in treatment animals at 3 months had nearly returned to baseline (BAS, 2.73 ± 0.70 mL/min/torr vs. 3-$month_{BLVR}$, 2.22 ± 0.68 mL/min/torr).

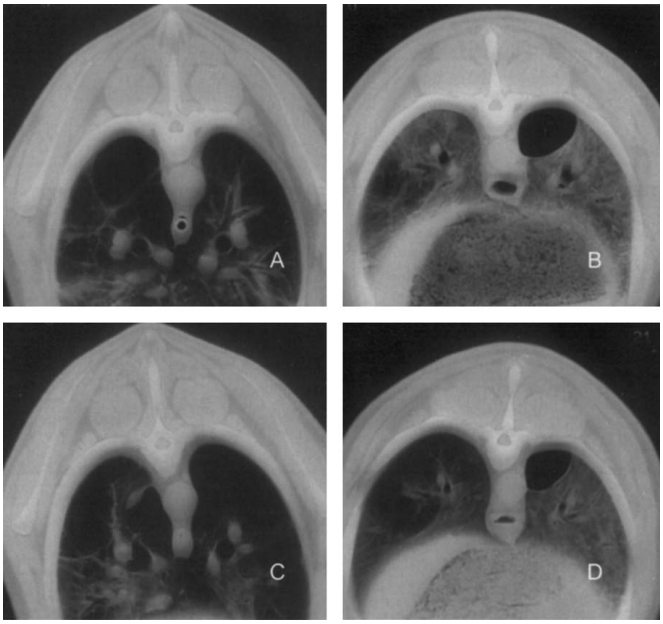

FIGURE 3. Serial CT scans of treated and control animals. A, BLVR animal at EMPH time point. B, Control animal at EMPH time point. Prominent bullae are visible. C, BLVR animal 3 months after treatment. Bulla has resolved. D, Control animal at 3 months. Prominent bulla is still present.

Radiologic Outcomes

Postpapain CT scans revealed heterogenous emphysema, with localized destruction at areas of papain injection. Large (>3 cm longest diameter), stable bullous lesions were observed in 1 animal from each experimental test group. CT guidance was used to deliver BLVR treatment to a dorsally located, 5.2-cm diameter lesion (Figs. 1A, B and Fig. 3A). By 1 month after BLVR treatment, this bulla was visibly reduced, and by 3 months this large lesion had completely resolved (Fig. 3B). The bulla was replaced by a well-organized, linear scar. By contrast, a similarly located dorsal bulla in a control animal showed minimal change in size over the 3-month follow-up (Figs. 3C, D).

Pathologic Outcomes

At necropsy, gross examination revealed multiple areas of collapse in the lungs of treatment animals. Histologic evaluation revealed heterogeneous emphysema with organized scar tissue at treatment sites. Heterogeneous emphysema without collapse or scar was seen in control animals. There was no evidence of abscess or pneumonia, and the remaining organs were grossly and microscopically normal in all animals.

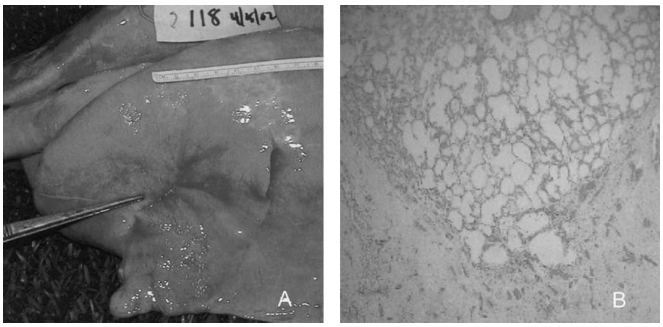

FIGURE 4. Pathologic findings in treated animal. A, Gross appearance of lung surface at the treatment site. Note the visible loss of lung volume. B, Microscopic appearance of treatment site showing atelectasis and scar adjacent to emphysematous lung (H&E, original magnification ×40).

DISCUSSION

We previously described the use of BLVR in a sheep model of homogeneous emphysema, demonstrating the safety and efficacy of the technique. The current study extends these observations to a model of heterogeneous emphysema that more closely mimics human disease. The combination of localized instillations of papain and papain nebulizations was used to produce heterogeneous emphysema with frank bulla formation in some animals. BLVR was safe and well tolerated in this model with no clinically significant adverse outcomes. Treatment resulted in a reduction in trapped gas and other physiologic improvements similar to those observed in the homogeneous emphysema model.[14,15] Gross and microscopic pathology demonstrated collapse and scar formation as intended at treatment sites. These findings confirm that BLVR using tissue engineering principles is a safe and potentially effective modality for treatment of both homogeneous and heterogeneous emphysema. Furthermore, site-directed BLVR treatment was used to target specifically and to reduce a frank bullous lesion in 1 animal. The possibility that BLVR could be used as a bronchoscopic alternative to surgical bullectomy warrants further exploration. Clinical trials of BLVR are currently underway to establish whether the salutary effects of BLVR can be reproduced in humans.

REFERENCES

1. Fishman A, Martinez F, Naunheim K, et al. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med*. 2003;348:2059–2073.
2. Patients at high risk of death after lung-volume-reduction surgery. *N Engl J Med,* 2001;345:1075–1083.
3. Utz JP, Hubmayr RD, Deschamps C. Lung volume reduction surgery for emphysema: out on a limb without a NETT. *Mayo Clin Proc*. 1998;73:552–566.
4. Elpern EH, Behner KG, Klontz B, et al. Lung volume reduction surgery: an analysis of hospital costs. *Chest*. 1998;113:896–899.
5. Ramsey SD, Sullivan SD, Kaplan RM, et al. Economic analysis of lung volume reduction surgery as part of the National Emphysema Treatment Trial. NETT Research Group. *Ann Thorac Surg*. 2001;71:995–1002.
6. Swanson SJ, Mentzer SJ, DeCamp MM Jr, et al. No-cut thoracoscopic lung plication: a new technique for lung volume reduction surgery. *J Am Coll Surg*. 1997;185:25–32.
7. Sabanathan S, Richardson J, Pieri–Davies S. Bronchoscopic lung volume reduction. *J Cardiovasc Surg (Torino)*. 2003;44:101–108.
8. Wantanabe Y. *LVRS with WBA*. Presented at the 12th World Congress for Bronchology and 12th World Congress for Bronchoesophagology; Boston, MA; 2002.
9. Toma TP. The flexible bronchoscopic approach to lung volume reduction. *Pneumologia*. 2001;50:97–100.
10. Toma TP, Hopkinson NS. Hillier J, et al., Bronchoscopic volume reduction with valve implants in patients with severe emphysema. *Lancet*. 2003;361:931–933.
11. Fann JI, Berry GJ, Burdon TA. Bronchoscopic approach to lung volume reduction using a valve device. *J Bronchol*. 2003;10:253–259.
12. Morrell NW, Wignall BK. Biggs T, et al. Collateral ventilation and gas exchange in emphysema. *Am J Respir Crit Care Med*. 1994;150:635–641.
13. Terry PB, Traystman RJ, Newball HH, et al. Collateral ventilation in man. *N Engl J Med*. 1978;298:10–15.
14. Ingenito EP, Berger RL, Henderson AC, et al. Bronchoscopic lung volume reduction using tissue engineering principles. *Am J Respir Crit Care Med*. 2003;167:771–778.
15. Ingenito EP, Reilly JJ, Mentzer SJ, et al. Bronchoscopic volume reduction: a safe and effective alternative to surgical therapy for emphysema. *Am J Respir Crit Care Med*. 2001;164:295–301.
16. Hoffman A, Henderson AC, Tsai L, et al. Physiologic responses of sheep to two different methods of papain exposure. *Inhal Toxicol*. 2003;15:761–780.
17. DuBois A, Botelho S, Bedell G, et al. A rapid plethysmographic method for measuring thoracic gas volume: a comparison with a nitrogen dilution method for measuring functional residual capacity in normal subjects. *J Clin Invest*. 1956;35:322–326.
18. Salazar E, Knowles J. An analysis of pressure volume characteristics of the lungs. *J Appl Physiol*. 1964;19:97–104.