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Early View

Research letter

External Radiofrequency as a Novel Extracorporeal Therapy for Emphysema

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Title:

External Radiofrequency as a Novel Extracorporeal Therapy for Emphysema

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Take home message:

In this study, we show in an animal model of emphysema that percutaneous application of radiofrequency energy in the form of electromagnetic waves improved lung compliance by selectively heating pulmonary emphysematous tissues and inducing mild fibrosis in the affected lung, while sparing normal lung regions. Radiofrequency treatment is a potential novel therapy for extracorporeal treatment of pulmonary emphysema.

COPD is characterized by the destruction of lung tissue resulting in alveolar tissue destruction, enlargement of alveolar spaces, poor gas exchange and airway collapse due to the loss of elastic recoil [1]. Lung volume reduction surgery (LVRS) is effective in reducing long term morbidity and mortality of patients with severe emphysema who have a predominance of upper lobe disease and are able to tolerate the surgical procedure [2, 3]. However, the financial cost and the peri-operative morbidity and mortality of the procedure have limited its application in clinical practice [4,5]. Here, we investigated the possibility of using external radiofrequency (RF) as a novel extracorporeal treatment for emphysema in a rat model of unilateral emphysema.

Thirty Sprague-Dawley male rats (7-8 weeks old) were subjected to 3 different conditions: saline (n=6), porcine pancreatic elastase (PPE) (n=12), and PPE+RF (n=12). Animals assigned to PPE and PPE+RF were instilled with PPE (100U/100g body weight) selectively into the left lung. Animals in the saline group were given an equal volume of saline (into the left lung) using the same method. Two weeks following the PPE treatment, the animals assigned to PPE+RF group were treated with RF therapy. The RF device consisted of three parts: an oscillator, an RF amplifier capable of generating 100W of energy and a matching network. After the animals were placed in a supine position under anesthesia, one electrode (2.0 x 2.0 cm) was placed on the lateral side of the chest wall and the other on the opposite chest wall, thus creating a "sandwich" to simultaneously expose both lungs to RF energy. To prevent skin burns, animals' chest fur was removed by an electric shaver and through a soft plastic tube, cold saline was applied at a constant rate to the chest wall during RF treatment. Three weeks following RF therapy, animals were sacrificed and the lungs were harvested for histology and lung compliance measurements. Lung compliance was measured by a water displacement method using Archimedes' principle [6]. To measure compliance in each lung, the bronchus to the contralateral lung was interrupted with a small clamp. A known volume of air was then added through a plastic tube, which resulted in changes in lung volume. Lung compliance was calculated as ΔVolume/ΔPressure [7]. For histology, the harvested lung with trachea was instilled with a solution containing 10% formalin at 20 cmH_2O pressure until both lungs were fully expanded. Thin slices were created by cutting uniformly in cross-sections from the most caudal position of the lung to its apex, equidistance apart. The slices were stained with hematoxylin and eosin (H&E) and scanned using the Aperio (Aperio Technologies; Vista, CA) scanning system at 40x. In accordance with the ATS/ERS guidelines on stereology, a systematic uniform random (SUR) sampling was performed on histological cross-sections of the lungs to determine mean airspace size (mean linear intercept, Lm) and extent of fibrosis (Ashcroft score [8,9]). A total of 10 images (image size = 1mm^2) were randomly extracted from all slides. Lm was calculated by the STEPanizer software [10] using line grids (line length 0.150 mm, 18 lines per image) on 10 randomly extracted images. Lung slices, which were stained with Masson's trichrome, were analyzed by a modified Ashcroft scoring system to evaluate the extent of fibrosis [9]. Each one mm square lung field was scored and the average score was calculated for each lung. This study was approved by the Animal Care Committee of the University of British Columbia (A10-0306, 0264, 0321). The care and handling of the animals were in accordance with the policies of the Canadian Council on Animal Care [11]. All results are expressed as mean value ± standard error (SEM) unless otherwise indicated. Data were analyzed using a Student t test (or a Mann–Whitney U test, when sample sizes were small and/or when the data did not approximate a normal distribution). All analyses were conducted using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

4 Out of 30 rats were euthanized post-PPE instillation owing to complications of PPE, which led to the following distribution of animals across the experimental groups: PPE (n=9), PPE+RF (n=11) and saline instillation controls (n=6). Five Weeks after PPE instillation, the left lung showed emphysematous changes on H & E staining, whereas the right lung demonstrated no significant emphysema and appeared similar to the lungs of animals in the saline control group (Fig. 1A). Lm of the left lung was increased with PPE instillation (179.8±10.4µm vs 128.4±17.4 µm, $p<0.001$; Fig. 1B). In the PPE group, the right lung also showed mild increases in Lm, consistent with very mild emphysema (117.3±10.3µm vs 99.6±13.9µm, $p=0.018$; Fig. 1B). The comparison of left and right lung showed significant increases in Lm of the left lung (which was exposed to PPE) compared with the right lung (which was not exposed to PPE) in the same rat (179.8±10.4µm vs 117.3±10.3µm, $p<0.001$).

PPE also significantly increased the compliance of the left lung compared with saline controls (155.9±16.7µl/cmH_2O vs 72.2±12.4µl/cmH_2O, $p=0.003$; Fig. 1C). PPE-instilled left lung showed mild fibrotic changes based on the modified Ashcroft scoring analysis; in contrast, the right lung demonstrated no significant fibrotic changes (0.31±0.16 vs 0.11±0.08, $p=0.013$; Fig. 1D).

The RF treated left lungs were less emphysematous as determined on histology compared to the left lungs of animals in the PPE group (Fig. 1A) which were not treated with RF (Lm of PPE+RF group, 159.5±30.5µm vs Lm of PPE group, 179.8±10.4 µm, $P=0.033$; Fig. 1B). RF significantly decreased the left lung compliance compared with the PPE only group (90.3±7.34 µl/cmH2O vs 155.9±16.70 µl/cmH2O, $p=0.012$; Fig. 1C). RF treated left lung showed mild fibrotic changes on the alveolar wall surface. RF treated left lung demonstrated a significant increase in the Ashcroft score compared with the left lung of animals in the PPE only group (1.21±0.30 vs 0.31±0.16, $P<0.001$; Fig. 1D). There were no significant changes in the right lung across the groups.

The total number of immune cells in the lungs were similar in PPE vs PPE+RF vs saline control groups (Fig. 1E).

To the best of our knowledge, this is the first report describing the potential therapeutic effects of external radiofrequency treatment for emphysema. It is widely known that emphysematous lungs have significantly reduced blood flow [12]. Therefore, we investigated the possibility of exploiting this physiological phenomenon to selectively over-heat emphysematous lungs and cause thermal injury while sparing healthy tissue with the cooling effect of the blood flow (which is termed the "heat sink" effect).

Pulmonary compliance is an important physiological abnormality in emphysema [13]. PPE-instilled left lung showed an increase in compliance, consistent with emphysema. With RF treatment, the emphysematous left lung showed a significant decrease in lung compliance suggesting potential physiological benefits. By contrast, RF therapy had no impact on the right lung, which did not demonstrate significant emphysematous changes. Together, these data suggest that RF therapy selectively modifies the lung compliance of emphysematous areas. These data are consistent with a case report by O'Meara et al, which described a patient whose lung function was improved by radiation therapy for lung cancer [14]. However, unlike conventional radiotherapy, which uses ionizing radiation, RF waves are non-ionizing and thus non-carcinogenic and safe for patients with COPD.

Because PPE induces an inflammatory reaction initially and then fibrosis later on [15], it was not surprising that we observed very mild fibrotic changes in left lung five weeks post-PPE instillation. RF treatment led to significantly increased fibrotic changes in the left lung, though the extent of fibrosis was still relatively mild. Collectively, these data suggest that RF therapy induces mild fibrotic changes in emphysematous lung, while sparing the normal lung. Importantly, at 5 weeks post-PPE instillation, there was no evidence of ongoing inflammation in the right or left lungs.

There are several limitations in the present study. First, although PPE-induced emphysema is a commonly used animal model of COPD [16], it does not perfectly mimic the human condition, which is largely caused by cigarette smoke and is characterized mostly by centrilobular and not panlobular emphysema. Second, although we successfully proved that RF therapy improved lung compliance of emphysematous lung using a rat model, we have not demonstrated an improvement in the functional status of these animals. Third, it is not certain whether the improvements in lung compliance related to RF therapy is permanent or temporary.

Notwithstanding these limitations, in this study, we have demonstrated that external RF therapy improves lung compliance of emphysematous lung by inducing mild fibrosis. Given the non-ionizing nature of RF waves, RF energy could be a novel intervention to reduce the burden of emphysema in COPD patients.

References

1. Hogg, J.C., *Pathophysiology of airflow limitation in chronic obstructive pulmonary disease.* Lancet, 2004. **364**(9435): p. 709-21.
2. Fishman, A., et al., *A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema.* N Engl J Med, 2003. **348**(21): p. 2059-73.
3. Naunheim, K.S., et al., *Long-term follow-up of patients receiving lung-volume-reduction surgery versus medical therapy for severe emphysema by the National Emphysema Treatment Trial Research Group.* Ann Thorac Surg, 2006. **82**(2): p. 431-43.
4. Naunheim, K.S., et al., *Predictors of operative mortality and cardiopulmonary morbidity in the National Emphysema Treatment Trial.* J Thorac Cardiovasc Surg, 2006. **131**(1): p. 43-53.
5. National Emphysema Treatment Trial Research, G., et al., *Patients at high risk of death after lung-volume-reduction surgery.* N Engl J Med, 2001. **345**(15): p. 1075-83.
6. Fahlman, A., et al., *Static inflation and deflation pressure-volume curves from excised lungs of marine mammals.* J Exp Biol, 2011. **214**(Pt 22): p. 3822-8.
7. Limjunyawong, N., et al., *Measurement of the pressure-volume curve in mouse lungs.* J Vis Exp, 2015(95): p. 52376.
8. Hsia, C.C., et al., *An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure.* Am J Respir Crit Care Med, 2010. **181**(4): p. 394-418.
9. Hubner, R.H., et al., *Standardized quantification of pulmonary fibrosis in histological samples.* Biotechniques, 2008. **44**(4): p. 507-11, 514-7.
10. Tschanz, S.A., P.H. Burri, and E.R. Weibel, *A simple tool for stereological assessment of digital images: the STEPanizer.* J Microsc, 2011. **243**(1): p. 47-59.
11. *The Canadian Council on Animal Care guidelines* Available from: https://www.ccac.ca/en/standards/guidelines/.
12. Chaouat, A., R. Naeije, and E. Weitzenblum, *Pulmonary hypertension in COPD.* Eur Respir J, 2008. **32**(5): p. 1371-85.
13. Papandrinopoulou, D., V. Tzouda, and G. Tsoukalas, *Lung compliance and chronic obstructive pulmonary disease.* Pulm Med, 2012. **2012**: p. 542769
14. O'Meara, P., et al., *Mechanisms of dyspnoea relief following radiation treatment in a patient with severe COPD.* Eur Respir J, 2011. **38**(3): p. 728-30.
15. Couillin, I., et al., *IL-1R1/MyD88 signaling is critical for elastase-induced lung inflammation and emphysema.* J Immunol, 2009. **183**(12): p. 8195-202.
16. Ghorani, V., et al., *Experimental animal models for COPD: a methodological review.* Tob Induc Dis, 2017. **15**: p. 25.

Figure legends

Fig. 1 A. H & E staining of lung tissue

Saline or porcine pancreatic elastase (PPE) was instilled only into left lung of each group. Obvious emphysematous changes were seen in left lung of PPE group.

Fig. 1 B: The effect of radiofrequency therapy on emphysema

Mean linear intercept (Lm) comparison (Mann–Whitney U test) on histological cross-sections of right and left lungs of animals treated with porcine pancreatic elastase (PPE) with or without radiofrequency (RF) therapy versus controls.

Fig. 1 C: The effect of radiofrequency therapy on lung compliance

Lung compliance was measured in lungs of animals treated with porcine pancreatic elastase (PPE) and with (or without) radiofrequency (RF) therapy and controls (Mann–Whitney U test); 5 cmH_2O to 10 cmH_2O of pressure was applied to each lung to generate these data.

Fig. 1 D: The effect of radiofrequency therapy on lung fibrosis

Lung fibrosis was determined semi-quantitatively using a modified Ashcroft score (Mann–Whitney U test).

Fig. 1 E: The effect of radiofrequency therapy on lung inflammation

The total number of immune cells were determined using microscopy (15x magnification) on 4 random fields of view per animal in all 3 groups (N=5 animals each in saline versus PPE versus PPE+RF therapy). The comparisons using Mann-Whitney U test showed no statistical significance.

Figure 1A

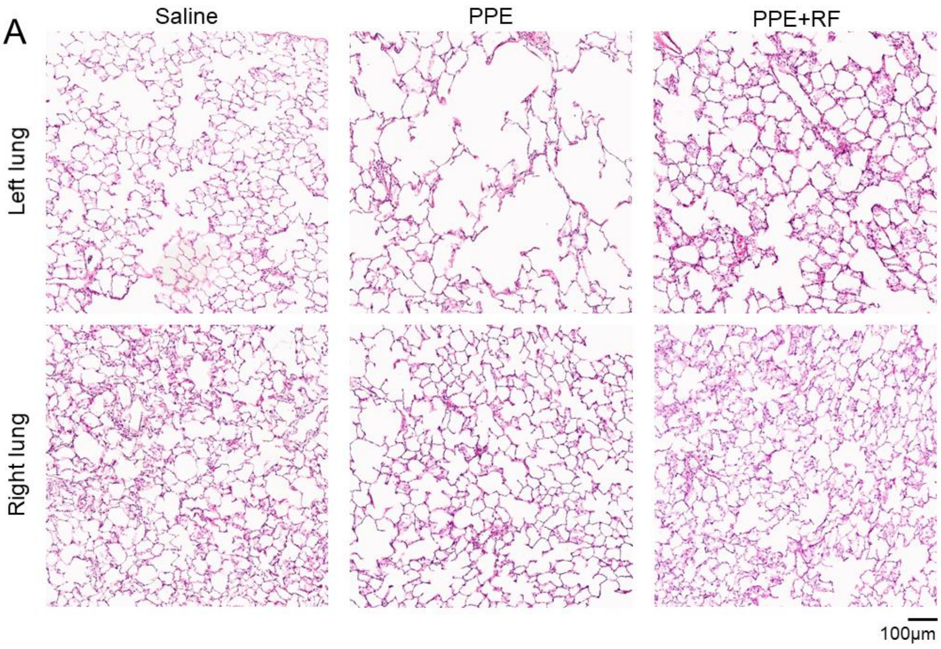
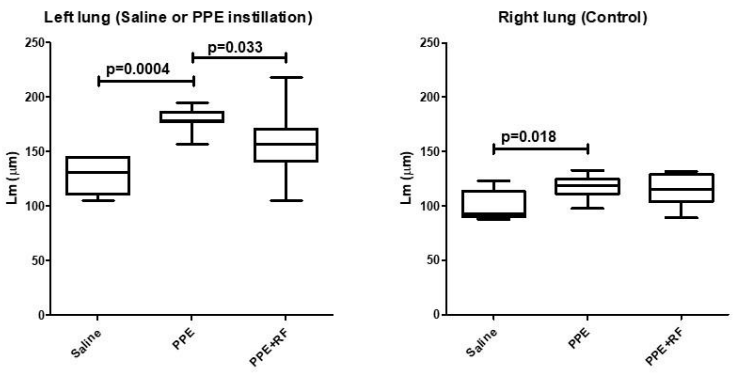


Figure 1B



Left lung (Saline or PPE instillation)
Right lung (Control)
p=0.0004
p=0.033
p=0.018
Lm (μm)
250
200
150
100
50
0
Saline
PPE
PPE+RF

Figure 1C

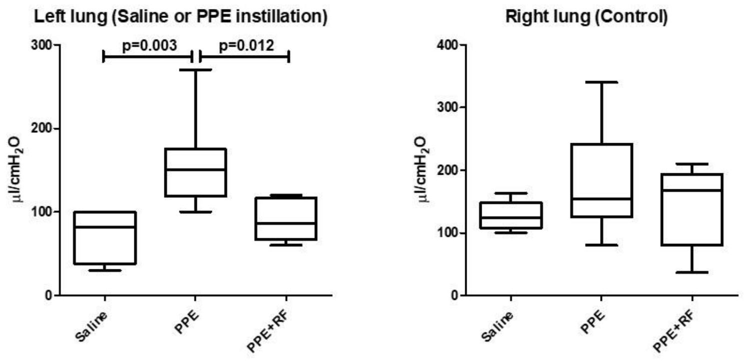
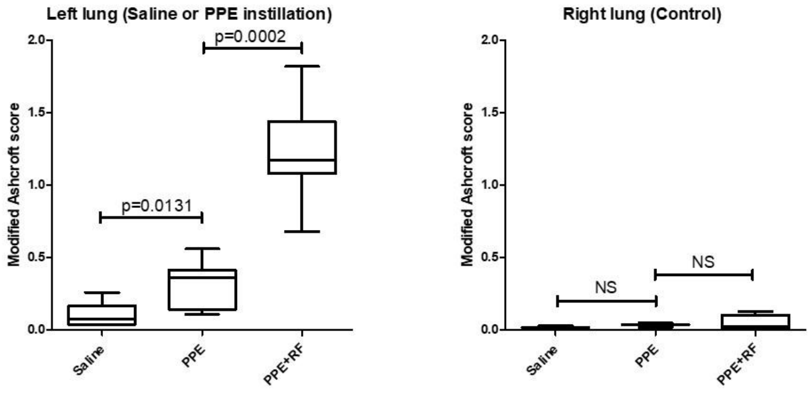

Left lung (Saline or PPE instillation)
p=0.003
p=0.012
µl/cmH2O
300
200
100
0
Saline
PPE
PPE+RF
Right lung (Control)
µl/cmH2O
400
300
200
100
0
Saline
PPE
PPE+RF

Figure 1D



Left lung (Saline or PPE instillation)
Right lung (Control)
Modified Ashcroft score
2.0
1.5
1.0
0.5
0.0
p=0.0002
p=0.0131
NS
NS
Saline
PPE
PPE+RF

Figure 1E

N=5 per group
Averaging 4 fields of view at 15X magnification