AGORA
RESEARCH LETTER

External radiofrequency as a novel extracorporeal therapy for emphysema

To the Editor:

COPD is characterised 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 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 perioperative 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.

30 Sprague Dawley male rats (7–8 weeks old) were subjected to three 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 (100 U per 100 g 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. 2 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 100 W of energy and a matching network. After the animals were placed in a supine position and under anaesthesia, one electrode (2.0×2.0 cm) was placed on the lateral side of the chest wall and another on the opposite chest wall, thus creating a "sandwich" to simultaneously expose both lungs to RF energy. To prevent skin burns, the animal's chest fur was removed using an electric shaver and cold saline was applied through a soft plastic tube at a constant rate to the chest wall during RF treatment. 3 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 haematoxylin and eosin (H&E) and scanned using the Aperio (Aperio Technologies, Vista, CA, USA) scanning system at 40×. In accordance with the American Thoracic Society/European Respiratory Society guidelines on stereology [8], a systematic uniform random sampling was performed on histological cross-sections of the lungs to determine mean airspace size (mean linear intercept, L_m) and extent of fibrosis (Ashcroft score [8, 9]). A total of 10 images (image size 1 mm^2) were randomly extracted from all slides. L_m 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 analysed using a modified Ashcroft scoring system to evaluate the extent of fibrosis [9]. Each 1-mm^2 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±SEM, unless otherwise indicated. Data were analysed using a 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, USA).

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External percutaneous application of radiofrequency (RF) energy improved lung compliance by selectively heating emphysematous tissues and inducing mild fibrosis. RF treatment is a potential novel therapy for extracorporeal treatment of pulmonary emphysema. https://bit.ly/2ZbWgSk

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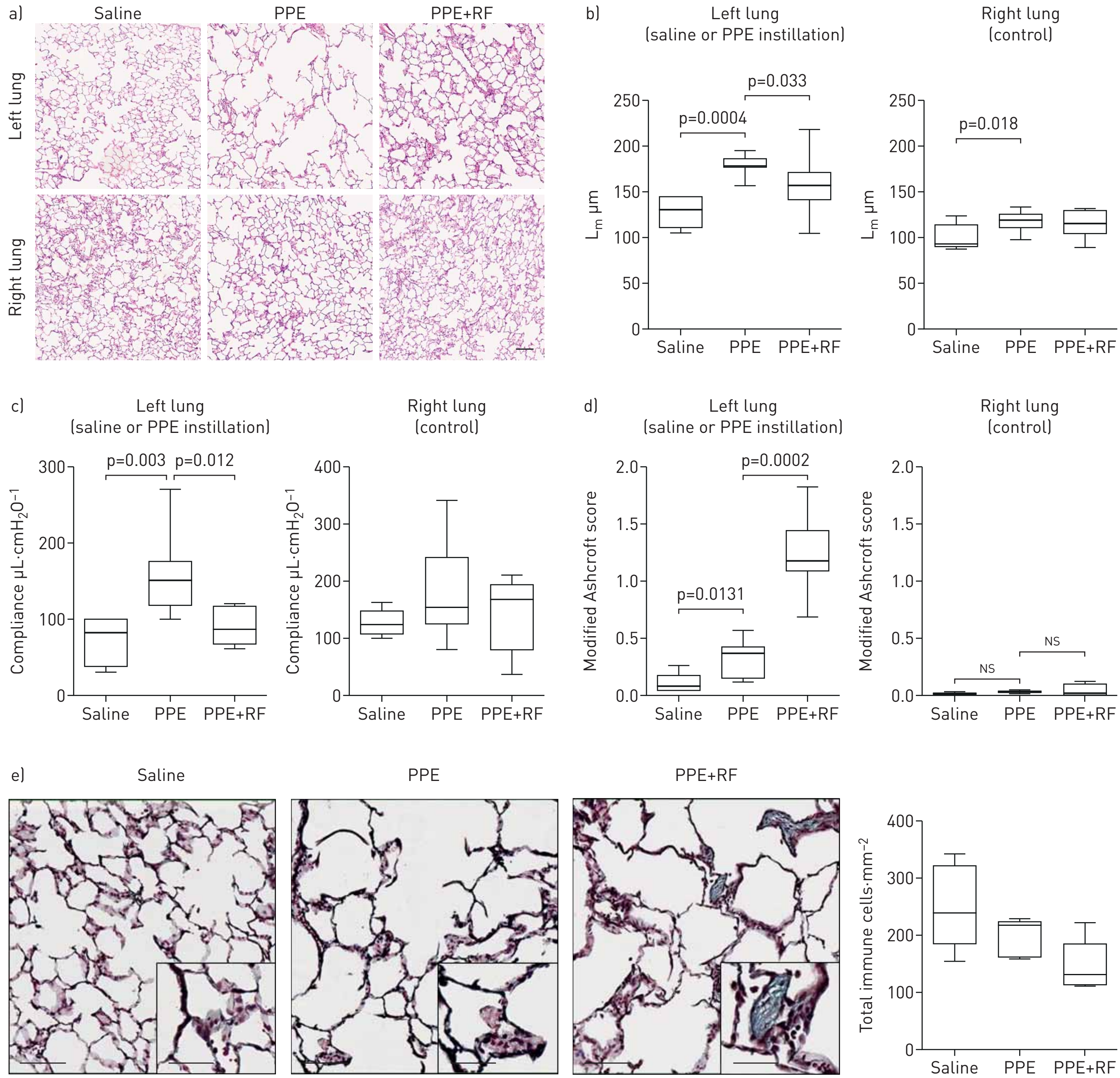

FIGURE 1 a) Haematoxylin and eosin staining of lung tissue. Saline or porcine pancreatic elastase (PPE) was instilled only into the left lung of each group. Obvious emphysematous changes were seen in the left lung of the PPE group. Scale bar=100 µm. b) The effect of radiofrequency therapy (RF) on emphysema. Mean linear intercept (L_m) comparison (Mann–Whitney U-test) on histological cross-sections of right and left lungs of animals treated with PPE with or without RF therapy *versus* controls. c) The effect of RF therapy on lung compliance. Lung compliance was measured in lungs of animals treated with PPE and with (or without) RF therapy and controls (Mann–Whitney U-test); 5–10 cmH_2O of pressure was applied to each lung to generate these data. d) The effect of RF therapy on lung fibrosis. Lung fibrosis was determined semi-quantitatively using a modified Ashcroft score (Mann–Whitney U-test). e) The effect of radiofrequency therapy on lung inflammation. The total number of immune cells were determined using microscopy (15× magnification) on four random fields of view per animal in all three groups (n=5 animals each in saline *versus* PPE *versus* PPE+RF therapy). The comparisons using Mann–Whitney U-test showed no statistical significance. Scale bars=100 µm (inset=50 µm).

Four out of 30 rats were euthanised 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). 5 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 (figure 1a). L_m of the left lung was increased with PPE instillation (179.8±10.4 µm *versus* 128.4±17.4 µm, p<0.001) (figure 1b). In the PPE group, the right lung also showed mild increases in L_m, consistent with very mild emphysema (117.3±10.3 µm *versus* 99.6±13.9 µm, p=0.018) (figure 1b). The comparison of left and right lungs showed significant increases in L_m 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±10.4 µm *versus* 117.3±10.3 µm, p<0.001).

In addition, PPE significantly increased the compliance of the left lung compared with saline controls (155.9±16.7 $\mu L \cdot cmH_2O^{-1}$ *versus* 72.2±12.4 $\mu L \cdot cmH_2O^{-1}$, p=0.003) (figure 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 *versus* 0.11±0.08, p=0.013) (figure 1d).

The RF-treated left lungs were less emphysematous as determined on histology compared to the left lungs of animals in the PPE group (figure 1a) which were not treated with RF (L_m of PPE+RF group 159.5±30.5 µm *versus* L_m of PPE group 179.8±10.4 µm, p=0.033) (figure 1b). RF significantly decreased the left lung compliance compared with the PPE-only group (90.3±7.34 $\mu L \cdot cmH_2O^{-1}$ *versus* 155.9±16.70 $\mu L \cdot cmH_2O^{-1}$, p=0.012) (figure 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 *versus* 0.31±0.16, p<0.001) (figure 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 *versus* PPE+RF *versus* saline control groups (figure 1e).

To the best of our knowledge, this is the first report describing the potential therapeutic effects of external RF 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.* [14], which described a patient whose lung function was improved by radiation therapy for lung cancer. However, unlike conventional radiotherapy, which uses ionising radiation, RF waves are nonionising and thus noncarcinogenic 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 5 weeks post-PPE instillation. RF treatment led to significantly increased fibrotic changes in the left lung, although 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 characterised 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 nonionising nature of RF waves, RF energy could be a novel intervention to reduce the burden of emphysema in COPD patients.

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