Airway gene-addition therapy for CF lung disease: strategies for improving gene transfer efficacy and longevity

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INTRODUCTION

- Airway gene-addition therapy is a mutation agonistic approach that has potential to treat CF lung disease.
- Gene-addition involves delivering normal *CFTR* gene copies to the airway cells to restore CFTR ion channel function.
- The airway epithelium acts as a physical barrier that impedes efficient gene transfer to the target cell types.
- Conditioning of the airway epithelium prior to gene vector delivery has previously been shown to improve vector transduction.
- The aim was to determine if our standard chemical conditioning or a novel mechanical method improved gene transfer in rats.
- To achieve this, the study was divided into two components:

1. Determine if LPC airway conditioning prior to lentiviral (LV) vector improves gene expression over short and long-term durations. This was assessed using bioluminescence imaging.
2. Assess epithelial conditioning methods and determine which approach results in the highest transduction levels. This was examined using LacZ histochemical staining.

METHODS

- Studies employed normal Sprague Dawley rats.
- All rats were anaesthetised for dosing procedures.

Chemical airway conditioning assessed over short and long-term

- Rats were endotracheally intubated.
- 25 µL 0.1% lysophosphatidylcholine (LPC) or phosphate-buffered saline (PBS) sham was delivered to the trachea.
- A LV vector carrying both *luciferase* and *green fluorescent protein* (GFP) reporter genes was used.
- Bioluminescence imaging was performed 1 week or 12 months following gene transfer (IVIS Lumina XRMS).

Comparison of airway conditioning methods

- The airway epithelium was conditioned using a novel mechanical approach followed by delivery of 50 µL LV-*LacZ* vector.
- LPC conditioning (as above) or LV vector only groups were performed for comparison.
- 1 week later the trachea and lung tissues were harvested for X-gal staining.

RESULTS

Luciferase gene expression following LPC airway conditioning or PBS-sham over short and long-term durations

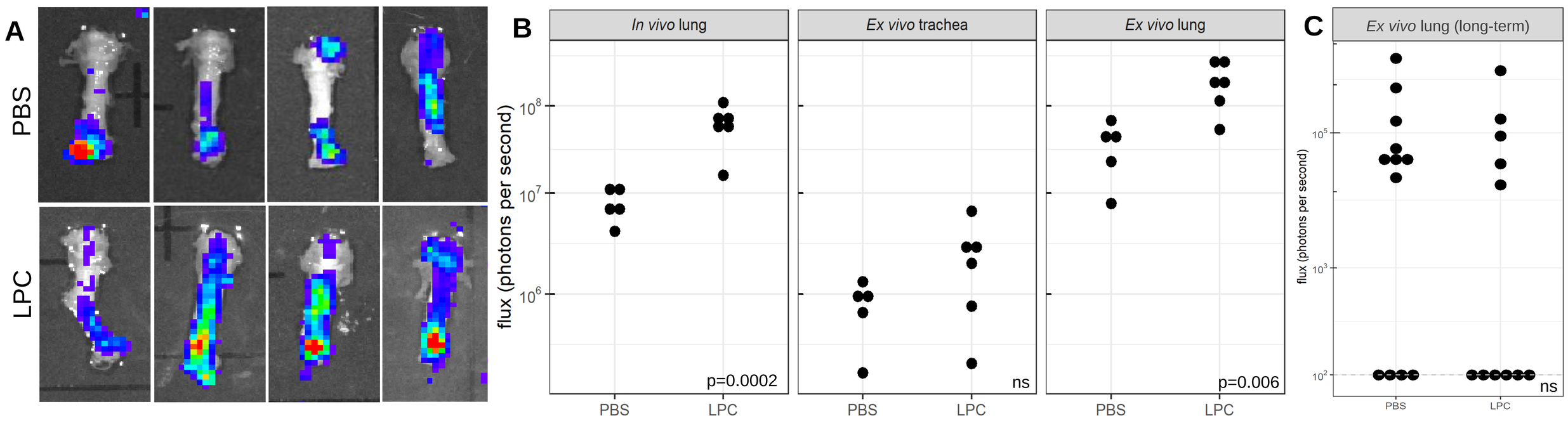

Figure 1: Bioluminescence flux acquired 1 week or 12 months following LV delivery in rats receiving LPC airway conditioning or PBS sham. **(A)** Examples of *ex vivo* trachea images from the 1 week study. **(B)** 1 week study showed that LPC results in significantly higher flux compared to PBS in the *in vivo* lungs. Upon excision and imaging of the tissues the *ex vivo* trachea showed no difference in flux between the LPC and PBS groups, while the *ex vivo* lungs had significantly greater flux in the LPC group when compared to PBS. Welch two sample t-test, n = 5-6 animals per group. **(C)** *Ex vivo* lungs indicated no significant difference between LPC and PBS-sham groups 12 months following LV delivery. Wald Chi-squared test, n = 11-12 animals per group.

Comparison of standard LPC and novel mechanical airway epithelial conditioning methods in the rat trachea

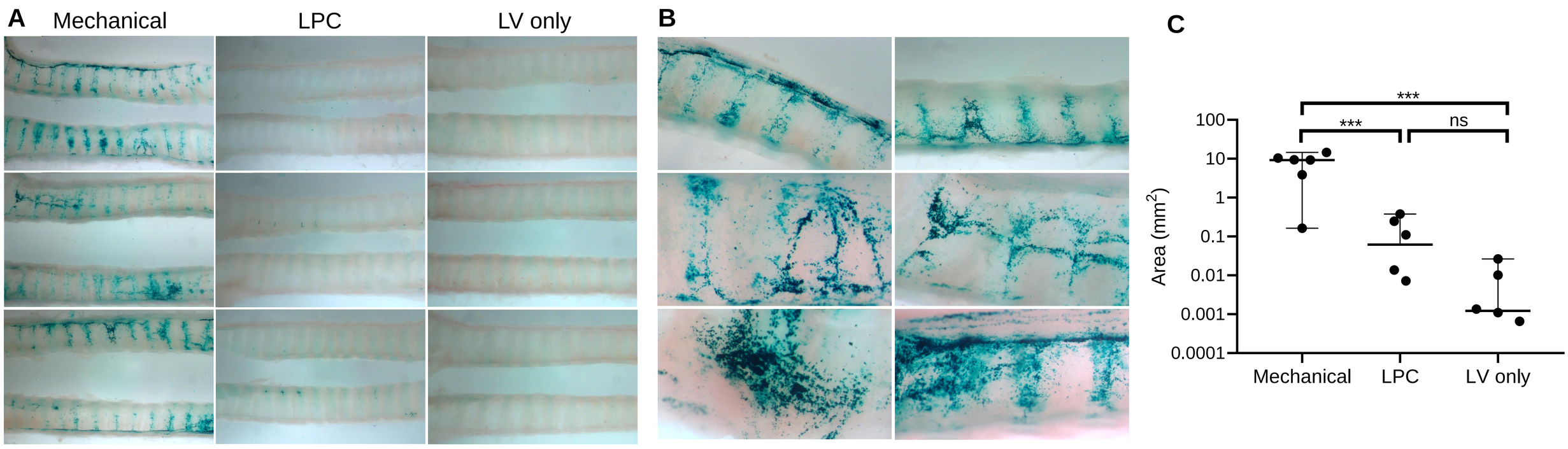

Figure 2: LacZ expression following use of a novel mechanical conditioning method, standard chemical conditioning with LPC, or LV vector delivery only. **(A)** Examples of *en face* trachea images show differing levels of LacZ expression depending on the method of airway conditioning. **(B)** High magnification *en face* blue stained images demonstrate varying patterns of LacZ staining produced from animals that received the novel mechanical conditioning method prior to LV delivery. **(C)** Digital quantification of LacZ positive staining area from *en face* images. The mechanical method demonstrated a significantly greater area of LacZ staining when compared to LPC conditioned animals (p=0.001) and LV only controls (p=0.0008). There was no significant difference in the area of LacZ staining between LPC-conditioned rats and those that received only LV vector (p=0.1). Graph indicates the median ± 95% CI, one-way ANOVA, Tukey's post-hoc test, n=6 rats per group.

CONCLUSIONS

- LPC conditioning improves gene expression levels in the lungs over short-term but not long-term durations. LPC does not appear to improve transduction levels in the trachea when compared to PBS-sham.
- The novel mechanical conditioning method substantially improves transduction levels in the trachea and may provide a new option for greatly improving airway LV gene-addition development.

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