SIX MONTHS LENTIVIRAL CORRECTION OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR GENE DEFECT IN CYSTIC FIBROSIS MICE

Patricia Cmielewski[1,3], Donald Anson[2] & David Parsons[1,3,4,5]

[1] Respiratory and Sleep Medicine, Women's and Children's Hospital, Adelaide, South Australia; [2]Gene Technology Unit, SA Pathology;
[3] Paediatrics, University of Adelaide, Adelaide; [4] Women's and Children's Health Research Institute, Adelaide, South Australia
[5] Centre for Stem Cell Research, University of Adelaide

Introduction

The assessment of functional genetic correction of the bioelectrical defect in cystic fibrosis (CF) mice nasal airway via transepithelial potential difference (TPD) measurements is technically challenging and can show high variability.

We examined the effects of our lentiviral (LV) gene therapy protocols over 6 months using a repeated-measures experimental design in CF mice.

Methods

Male and female CF^{tm1unc} mice were instilled nasally with either PBS (control) or lysophosphatidylcholine (LPC) one hour prior to delivery of an LV vector containing the CFTR gene. An additional group of CF mice received LPC with an empty vector (LV-MT).

Nasal TPD measurements were performed under domitor/ketamine anaesthesia at 1 week, and at 1, 3, and 6 months after treatment, using standard basal, basal+amiloride (amil:10^{-4}M) level and low-chloride+amiloride Krebs solutions (Fig. 1). The ΔPD, an index of functional CFTR correction, was calculated as the low-chloride TPD minus the basal TPD.

Results

In mice that received LPC/LV-CFTR a significant correction of ΔPD towards normal values was seen at 1 week. The correction has persisted for at least 6 months to date (*$p<0.05$, RM ANOVA, n =6-12/group) (Fig. 2). There was no correction produced by (and no difference between) the two control groups PBS/LV-CFTR and LPC/LV-MT at all time points. There was no effect of LPC alone on TPD measures (data not shown).

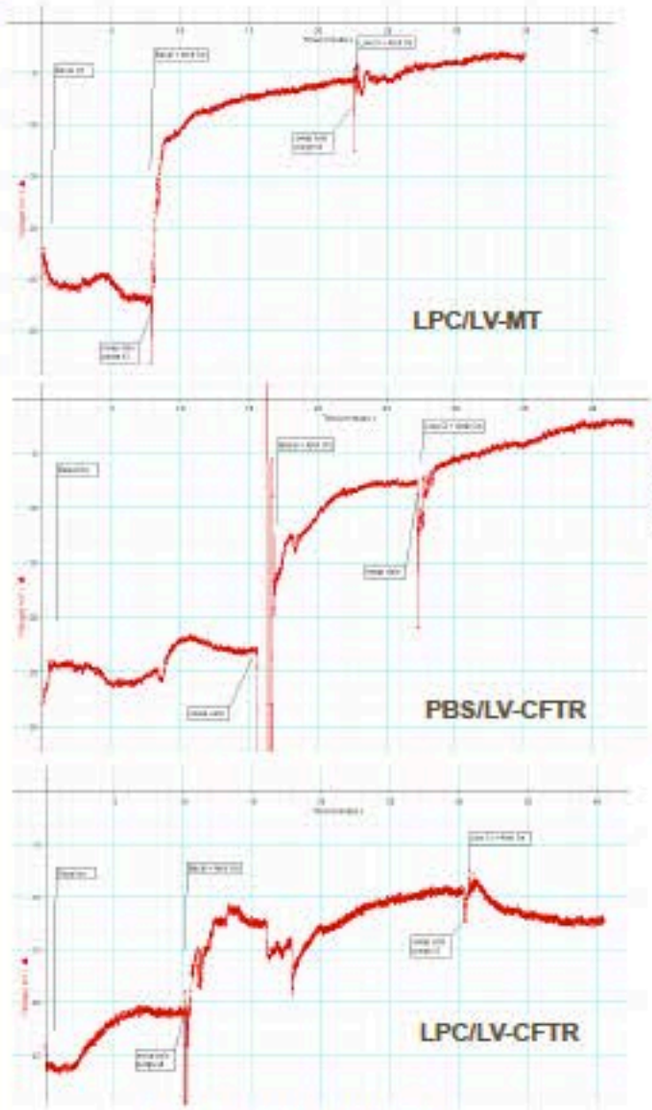

Fig. 1. Nasal TPD trace

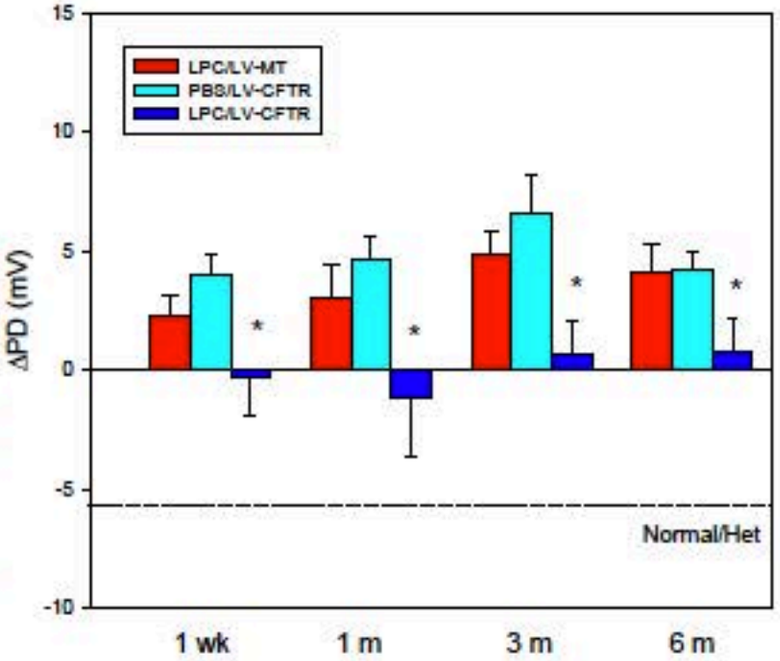

Fig. 2. ΔPD over time

Conclusion

Partial (~ 40%) sustained correction of the CFTR bioelectric defect is shown in CF mouse nasal airway after LPC/LV-CFTR LV vector treatment, with the duration of CFTR gene transfer reported now extended to at least 6 months after a single brief dose. Repeated-measure study designs can be successfully applied to CF transgenic mice using these anaesthesia and dosing protocols over at least 6 months without mortality associated with anaesthesia or TPD procedures.

Acknowledgements

NH&MRC and philanthropic donors (www.Cure4CF.org).