Aerosol delivery of concentrated PEI formulations to the sheep lung

Lee Davies
Gene Medicine Research Group
Oxford University
& United Kingdom Cystic Fibrosis Gene Therapy Consortium

Polyethylenimine

- Highly cationic polymer
- Readily complexes with pDNA
- Potent GTA *in vitro* and *in vivo*
- Range of PEI polymers
- 25kDa branched PEI

- Introduce functional CFTR gene
- Ciliated epithelial cells
- Topical aerosol delivery of GTAs
Aerosol delivery of non-viral GTAs

- Generate GTA aerosols
- Clinical nebulisers
- Shear degradation of pDNA
- Some GTAs can protect pDNA
- 25kDa branched PEI

- *In vivo* models

Aerosol delivery to the mouse lung

- Mouse lung aerosol model
- Rapidly assess formulations
- Whole body exposure
- Aqueous DNA/PEI formulations
- N:P ratio of 10:1
- Clinical jet nebuliser

- Luciferase reporter
Aerosol delivery of PEI complexes

- Dose dependent lung expression
- Maximum concentration 0.5mg/ml
- Compares poorly to other GTAs
- Clinical viability
- Estimate require >50mg per patient
  - Delivery time
  - Delivery volume
- Higher concentrations required
- Ultrafiltration

Ultrafiltration of DNA/PEI formulations

- Concentrate standard formulations
- Stirred ultrafiltration cell
- 100kDa nitrocellulose membrane
- Concentrations up to 10mg/ml
- No precipitation
- No DNA in filtrate
- “Free” PEI removed during filtration
- Concentration effects
Physical characteristics of cPEI formulations

Little effect of concentration upon physical characteristics

Concentrated PEI in the mouse lung

BALB/c mice
n = 8
10ml aerosol
100μl instilled
Concentrated PEI in the mouse lung

- BALB/c mice
- n = 8
- 10ml aerosol
- 100μl instilled

PEI toxicity in the mouse lung

- 1mg/ml Instillation
- 8mg/ml Aerosol

Mouse lung sections
H&E (x40)
cPEI formulations in vivo

- **Mouse lung model**
  - Good gene expression from cPEI aerosols with minimal toxicity
  - Contrast to instillation
  - Delivery methodology important in expression and toxicity

- **Large animal aerosol delivery model**
  - Sheep lung
  - More clinically relevant delivery and expression model

---

Sheep aerosol model

- Suffolk cross ewes
- Anaesthetised
- Intubated
- Negative pressure ventilation
- 3 breath-actuated nebulisers

- 20ml aerosol
- 1 hr delivery time
Sheep aerosol delivery - gene expression

Equivalent expression with reduced volume and delivery time

Sheep aerosol delivery - BAL neutrophils

cPEI aerosol formulations exhibit lower toxicity
Summary

• Ultrafiltration reliable method to concentrate PEI formulations
• Improved clinical viability
• Relatively high expression in absence of significant toxicity

• 25kDa PEI very basic vector
• Encouraging results
• Novel synthetic polymers
• Suitability for aerosol delivery

Acknowledgements

• Oxford University
  Steve Hyde
  Deborah Gill
  Ian Pringle
  Stephanie Sumner-Jones
  Anna Lawton
  Hazel Painter
  Anusha Varathalingam

• Edinburgh University
  Gerry McLachlan
  David Collie

• Imperial College London
  Chela Nunez
  Rebecca Coles
  Anne-Marie Green