Characterisation of Aerosol Delivery of Lipid/DNA Formulations in Preparation for Gene Therapy Clinical Studies

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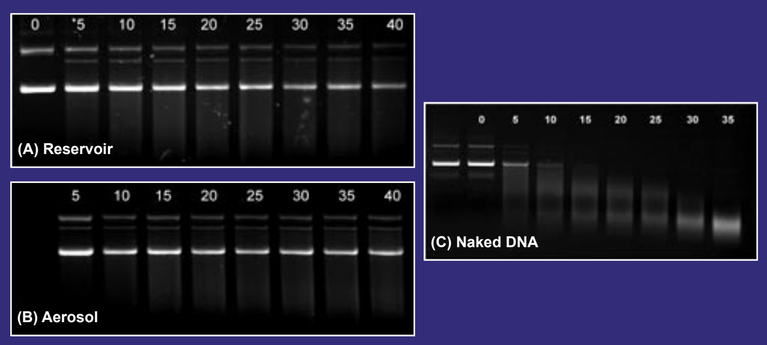
OVERVIEW

- Viral and non-viral gene transfer agents (GTAs) are being developed for treatment of CF lung disease.
- The UK CF Gene Therapy Consortium has compared the performance of many GTAs in the mouse and sheep lung.
- The animal model and delivery method chosen for evaluation are crucial, as lung delivery of a bolus of fluid via tracheal instillation or nasal sniffing can report misleading toxicity and efficiency.
- The UK CF Gene Therapy Consortium has selected GL67A cationic liposomes complexed with plasmid DNA for clinical evaluation after aerosol lung delivery.
- The clinical nebuliser system used previously for aerosol delivery of pDNA/GL67A formulation to CF patients has been discontinued.
- Five nebulisers were evaluated for their compatibility with the formulation and their aerosol output characteristics.

ANIMAL MODEL

- Plasmid DNA expressing Luciferase (pCIKLux) complexed with GL67A liposomes (8 mM:6 mM in sterile water).
- Whole body aerosol chamber containing BALB/c mice (25mg pDNA; n=6/group).
- The following commercially available jet nebulisers were tested:
  - Pari LC+
  - Pari Sprint
  - Pari Sprint Junior
  - Pari Sprint Star
  - Trudell AeroEclipse II

FORMULATION STABILITY

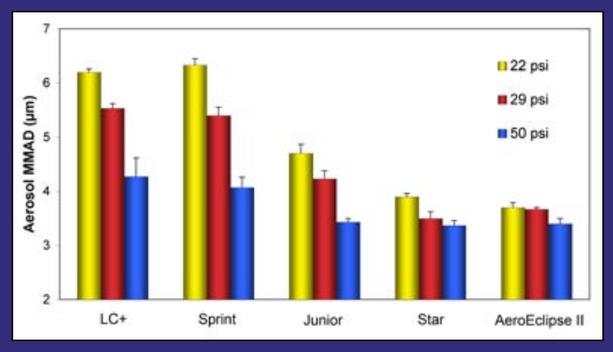


- The five test nebulisers were used to spray the pDNA/GL67A formulation.
- Aliquots of nebulised material were collected from the reservoir (A) and after aerosol delivery (B), and subjected to agarose gel electrophoresis.
- Nebulisation of naked (non-complexed) pDNA resulted in degradation after as little as 5-10 minutes of nebulisation.
- Complexing pDNA with lipid preserves formulation integrity after aerosol delivery with all five nebulisers tested.

AIM

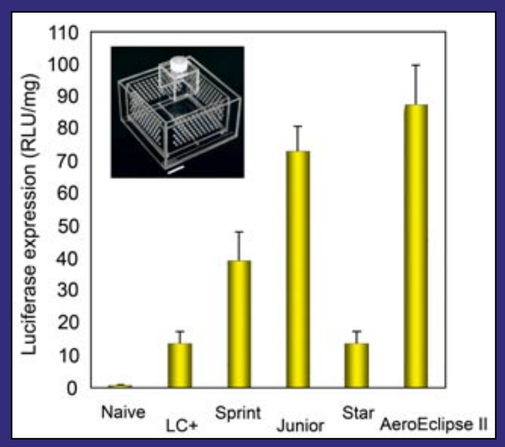
- To select a nebuliser for optimal aerosol delivery of pDNA/GL67A liposome complexes to the lungs of CF patients.

AEROSOL DROPLET SIZE



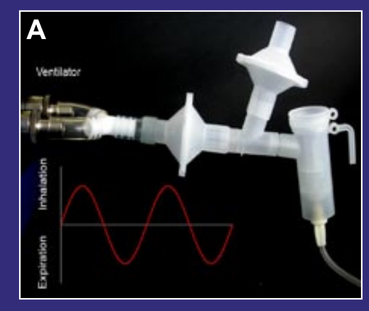
- The aerosol droplet mass median aerodynamic diameter (MMAD) suitable for delivery to the small airways of the CF lung is estimated to be 2-4 µm.
- The MMAD was measured for each nebuliser following inertial impaction of aerosol in a chilled (7-8˚C) Next Generation pharmaceutical Impactor (NGI) operating at 15 L/min.
- The MMAD for each nebuliser was dependent upon the operating pressure with the smallest droplets produced at 50 psi.
- The Sprint Junior (3.43 ± 0.07 µm), Sprint Star (3.37 ± 0.09 µm) and AeroEclipse II (3.40 ± 0.1 µm) generated suitably sized droplets.
- The Sprint Star nebuliser was prone to “sputtering” with pDNA/GL67A formulations and was excluded from further studies.

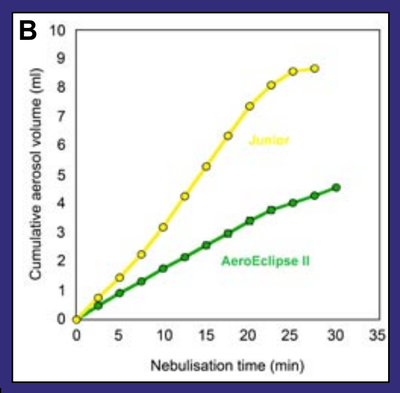
REPORTER GENE EXPRESSION

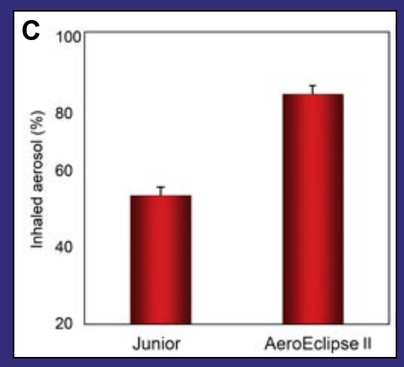


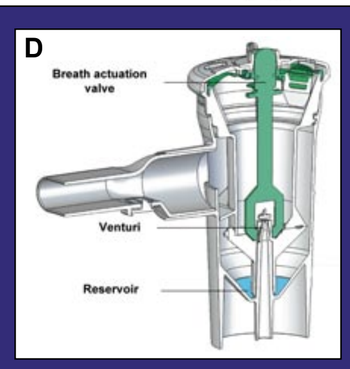
- The five test nebulisers were used to deliver the pDNA/GL67A formulation (10ml; 25mg pDNA) to mice (n=6) via the whole body exposure chamber.
- Luciferase reporter expression was measured in homogenised lung tissue and expressed as Relative Light Units /mg.
- All nebulisers were capable of generating detectable reporter gene expression.

AEROSOL DELIVERY RATE









- The aerosol delivery rate (nebuliser output) was measured with our pDNA/GL67A formulation, to confirm clinical acceptability.
- A human breath simulator (with a sinusoidal breathing pattern at 15 breaths/mi; inspiratory : expiratory ratio of 1:1; tidal volume 500 ml) was used (A).
- The Sprint Junior nebuliser was faster than the AeroEclipse II nebuliser indicating a 1-2h delivery time for clinically relevant (50mg dose) (B).
- The efficiency of the breath-actuated AeroEclipse II was significantly higher (83 ± 2% compared with 55 ± 1%) as determined by collection of aerosol on the inspiratory arm of the breathing circuit (C).
- Both the Sprint Junior and AeroEclipse II nebulisers would be suitable, but the AeroEclipse II was selected for clinical studies (D).

CONCLUSIONS

- pDNA/GL67A formulation can be aerosolised with only minimal loss of plasmid integrity and generated aerosols with MMAD suitable for human delivery.
- The breath actuated Trudell AeroEclipse II jet nebuliser has been selected for the aerosol delivery of the pDNA/GL67A formulation to CF patients.

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