Bactericidal Effects of Antibacterial Perfluorocarbon Ventilation

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Summary of Presentation

I. Background
   I. Need for improved treatment
   II. Review of liquid ventilation

II. Methods
   I. In vivo
   II. In vitro

III. Results

IV. Conclusions
Background
Background: Epidemiology

• Respiratory infection results in 500,000 hospitalizations, 110,000 deaths, and more than $13 billion in hospital costs per year

• Improved treatment is needed for cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and bronchiectasis patients with a bacterial infection
  – CF and COPD feature a change in mucus rheology that impairs mucociliary clearance, frequently leading to chronic infection and inflammation
  – Respiratory infections present in 55-60% of COPD exacerbations
  – 90% of US CF patients ultimately die of a respiratory infection
A Chronic Cycle of Infection

- Inflammation
- Airway damage
- Mucous secretions
- Inability to clear mucous
- Infection
Background: Current Treatments

- Current treatments utilizing aerosolized and intravenous antibiotics have several shortcomings
  - IV antibiotics must be administered at high levels to achieve ideal concentrations at site of infection, often increasing risk of systemic toxicity
  - Aerosolized antibiotics require narrow range of particle size to reach peripheral airways
  - Aerosolized delivery cannot reach the poorly ventilated, most diseased lung regions
Background: APV

- Antibacterial Perfluorocarbon Ventilation (APV) is a novel approach to pulmonary drug delivery that addresses the shortcomings of current treatment.
- Lungs are filled with an emulsion of tobramycin in PFC and then ventilated for <2 hours.
- Emulsion is composed of PFC, aqueous tobramycin and fluorosurfactant.
- Emulsion allows gas exchange due to PFC’s high solubility with O₂ and CO₂.
- APV can improve current treatment in several ways:
  - More uniform antibiotic distribution throughout the lungs.
  - Removal of infected mucus.
  - Anti-inflammatory properties inherent to PFCs.

![Figure 1: Airway depicting forces acting upon mucus. \( \tau_i \) and \( \tau_e \) are the shear stresses during inspiration and expiration, \( \sigma \) is the surface tension resisting mucus deformation, and \( F_B \) is the buoyant force due to the density difference between PFC and mucus.](image)
Methods
Methods: *In vivo*

**Animal Experiments**

- **Inoculation**
  - 200 µL inoculum containing approximately 10^6 colony forming units (CFU) of *P. aeruginosa* intra-tracheally delivered to each rat under sedation
  - Inoculum prepared by encasing mid-log growth *P. aeruginosa* in synthetic sodium alginate
### Treatment Schedule

<table>
<thead>
<tr>
<th>Day</th>
<th>Aerosolized</th>
<th>APV (large volume)</th>
<th>APV (small volume)</th>
<th>Negative Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Rats sedated and intra-tracheally inoculated with <em>Pseudomonas aeruginosa</em> ~10⁶ CFU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>No treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AM: aerosolized (15 mg/kg)</td>
<td>AM: large vol APV (15 mg/kg)</td>
<td>AM: small vol APV (15 mg/kg)</td>
<td>Anesthetized without treatment</td>
</tr>
<tr>
<td></td>
<td>PM: aerosolized (2.5 mg/kg)</td>
<td>PM: aerosolized (2.5 mg/kg)</td>
<td>PM: aerosolized (2.5 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AM: aerosolized (2.5 mg/kg)</td>
<td></td>
<td></td>
<td>Anesthetized without treatment</td>
</tr>
<tr>
<td></td>
<td>PM: aerosolized (2.5 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AM: aerosolized (2.5 mg/kg)</td>
<td></td>
<td></td>
<td>Anesthetized without treatment</td>
</tr>
<tr>
<td></td>
<td>PM: aerosolized (2.5 mg/kg)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>Rats euthanized, blood sample taken, and lungs harvested aseptically</td>
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</tbody>
</table>
APV Treatments

**Large Volume Instillation**
- 15 mL/kg instilled
- 0.1% aqueous volume
- 1000 mg aqueous tobramycin per mL H₂O
- 1 mg tobramycin per mL of emulsion

**Small Volume Instillation**
- 3 mL/kg instilled
- 0.5% aqueous volume
- 1000 mg aqueous tobramycin per mL H₂O
- 5 mg tobramycin per mL of emulsion
Methods: *In vivo*

**Necropsy and Tissue Analysis**
- All rats were euthanized following three days of treatment (five days post-inoculation)
- The right lung was harvested aseptically and homogenized in sterile saline
- Homogenized tissue was serially diluted and cultured on cetrimide agar (a *P. aeruginosa* selective growth medium) in order to quantify bacterial load
Bacterial Cultures

Figure 1: Bacterial load remained in the lungs post-treatment

Figure 2: The treatment successfully killed all infection in the lungs
Methods: *In vitro*

- **Biofilm Growth**
  - Biofilms were grown on plastic pegs by incubating a solution of mid-log growth *P. aeruginosa* in a 96-well plate with a peg lid for 18 hours.

- **Exposures**
  - Biofilm-coated pegs were then submerged in new wells containing one of five exposure mediums (preoxygenated emulsion, preoxygenated PFC, unoxygenated PFC, unoxygenated emulsion, or growth medium) for a period of 2 hours.
  - Preoxygenated groups were continually oxygenated throughout the exposure.

- **Assessing Bacterial Growth**
  - Following exposure, adherent biofilms were centrifuged off pegs into new 96-well plates containing sterile growth medium.
  - Bacterial growth was measured via change in optical density (600 nm) over
Methods: *In vitro*

Peg lids are incubated in a 96-well plate that is filled with a bacterial suspension for 3-4 hours.

Biofilms on the peg lid are removed from the bacterial suspension.

The peg lid is then placed in a new 96-well plate containing the desired exposure media.

Biofilms are centrifuged and the peg lid are removed from the exposures.

The biofilms are placed in fresh growth medium and optical density is measured. The plates are then incubated for six hours and optical density is measured again.
Results
Results: *In vivo*

**Bacterial Load Remaining after Treatment with Large Volume APV**

- pAPV [15 mL/kg] (n=5)
- Aerosolized (n=3)
- Untreated (n=4)
Results: \textit{In vivo}
Results: *In vitro*

**Biofilm Exposure Assay**

<table>
<thead>
<tr>
<th>Exposure Medium</th>
<th>Difference in Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSB + 1% Glu</td>
<td>0.44 (±0.05)</td>
</tr>
<tr>
<td>Unoxy PFC</td>
<td>0.78 (±0.10)</td>
</tr>
<tr>
<td>Oxy PFC</td>
<td>0.58 (±0.08)</td>
</tr>
<tr>
<td>Unoxy Emulsion</td>
<td>0.12 (±0.03)</td>
</tr>
<tr>
<td>Oxy Emulsion</td>
<td>0.06 (±0.02)</td>
</tr>
</tbody>
</table>
Conclusions
Animal Experiments

- Small volume APV results in lower remaining pulmonary bacterial load relative to aerosolized and large volume APV treatments.
- We believe small volume APV performs better due to decreased volume of the PFC phase:
  - PFC alone appears to promote bacterial growth (see biofilm exposure results), likely due to high O2 content.
  - Over time, it is likely that the aqueous, drug-containing droplets will separate from the PFC phase and coalesce before all of the PFC is blown off, potentially leaving neat PFC in contact with the bacteria.
  - This likely occurs to a greater degree during large volume APV.
- Although more work must be done to optimize treatment, we have shown that APV is a viable means of pulmonary drug delivery with the potential for improvement over current treatments.
Biofilm Exposures

• The emulsion is capable of inhibiting biofilm growth in an *in vitro* setting
• Neat PFC promotes more bacterial growth than standard growth medium
• Oxygen content does not have a statistically significant effect on bactericidal ability of the emulsion