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Bactericidal Effects of Antibacterial Perfluorocarbon Ventilation

Presentation by Katy Graham

Work done under the supervision of Ryan Orizondo, MSE

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I. Abstract

Current treatment of lower respiratory infections is hindered by poor antibiotic distribution throughout the lung. Antibacterial perfluorocarbon ventilation (APV) is a treatment method in which the lungs are filled with an emulsion containing a disperse phase of aqueous antibiotics within perfluorocarbon (PFC) liquids. The *in vitro* portion of this study assessed the effect of emulsion oxygen content on bactericidal ability. *P. aeruginosa* biofilms were exposed to oxygenated or unoxygenated emulsion, PFC, or growth medium for two hours, after which their viability was assessed. During *in vivo* experiments, the effectiveness of APV treatments with small (APV-S = 3 mL/kg) or large (APV-L = 15 mL/kg) lung fill volumes was assessed relative to aerosolized delivery using a rat respiratory infection model. Forty-eight hours following intratracheal delivery of *P. aeruginosa,* rats underwent a three-day treatment regiment in which an initial loading dose of tobramycin (15 mg/kg) was delivered via APV-S, APV-L, or aerosolized delivery. Following the loading dose, all rats received an aerosolized maintenance dose (2.5 mg/kg) every 8-12 hours. Five days post-inoculation, rats were euthanized and the remaining pulmonary bacterial load assessed. *In vitro* results have shown that oxygen content of the emulsion has no significant effect on the bactericidal ability. *In vivo* results have shown that APV utilizing small fill volumes decreases remaining bacterial load relative to aerosolized delivery, with a mean bacterial load of 4.2E2 and 7.7E3 CFU/mL for APV-S and aerosolized delivery, respectively. APV-L showed no benefit relative to aerosolized delivery.

II. Significance

Respiratory infections are one of the most prevalent causes of mortality and illness in the world.

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Especially in patients with cystic fibrosis or chronic infections, the inability of current treatments to completely clear an infection causes immeasurable an cost to society, including 500,000 hospitalizations, 110,000 deaths, and $13 billion in hospital related fees annually [1,2]. There exists a persistent cycle that many of these patients experience, starting with infection, leading to inflammation, which yields increased airway mucous secretions, and a resulting inability to excrete this mucous, which allows the bacteria to multiply and the infection to endure.

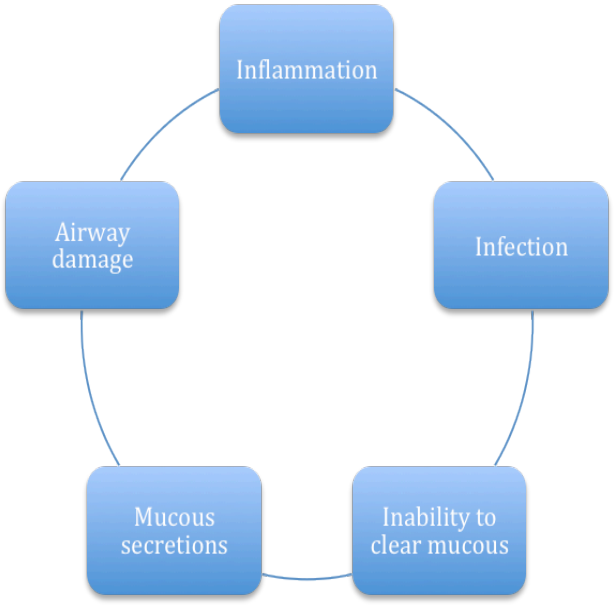

Figure 1: The chronic cycle of infection.

Bronchiectstasis, a chronic dilation of the airways, can occur, which in total costs $630 million per year (bronchiectstasis is also caused by HIV, COPD, etc.) [3,4]. Currently, intravenous and aerosolized antibiotics are used to treat these infections. Both are not efficient in clearing the bacteria. Intravenous antibiotics cannot reach a high enough concentration in the lungs due to a low concentration gradient by the time the blood circulates to the respiratory system. If you increase the

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concentration injected into the blood stream to solve this problem, the result can be toxic. To prevent this outcome, most doctors prescribe nebulized antibiotics. However the medicine cannot reach the deeper diseased portions of the lungs via a simple inhalation of antibiotics and the size of the antibiotic particle plays a large role in how well it can be absorbed into the alveoli [5,6]. Much of the antibiotic can be exhaled, which may also lead to drug resistant bacteria in the patient [7]. To ameliorate these issues, we propose to use liquid ventilation to deliver antibiotics directly to the infected portions of the lungs. The drug will be more evenly distributed, better adapted to remove mucous, and able to reduce inflammation due to natural properties of the liquid. We use tobramycin emulsified in perfluorocarbon liquid- a highly researched compound that can hold large amounts of oxygen and carbon dioxide gas. Perfluorocarbons, or PFCs, are highly useful for accessing the hard to reach areas of the airways and can more evenly coat antibiotics throughout the lungs [8]. Our methods have been adapted from previous experiments, but our biotechnology is novel.

III. Scope

In this experiment, we were testing the efficacy of APV in small rat models to determine how well the process can kill *Pseudomonas aeruginosa*. Our results are limited to the anatomy of rats, and we cannot definitely conclude how APV would work in humans. We would like to expand our trials to rabbits or sheep, which are more similar to humans, but even the results from this would not allow us to make conclusions about efficacy in human trials. Rats in our study will underwent a combined APV and aerosolized treatment plan, and their lungs and blood were be analyzed to determine how much of the *P. aeruginosa* infection was killed.

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IV. Methodology

1. Our current experiment's aim is to compare partial Antibacterial Perfluorocarbon Ventilation (pAPV) with aerosolized delivery of antibiotics in a rat respiratory infection model. We prepare and inoculate rats on Day 0 with *Pseudomonas aeruginosa* and leave them untreated for two days. On the morning of Day 2, we treat the experimental group with either small volume or large volume APV, give another group aerosolized antibiotics, and give the negative control group sham sedation. For the experimental group, all rats are anesthetized and intubated for the delivery of the liquid emulsion. Intravenous access is gained via the tail and heart rate and O2 saturation level are recorded throughout the experiment. Once intubated, the emulsion is instilled and the rat is connected to an oxygen ventilator to allow for oxygen exchange similar to that of normal breathing. Once the rat is ambulatory, we put it in an oxygen chamber and allow the perfluorocarbon liquid to evaporate from the lungs. In the aerosolized group, we anesthetize and intubate the rat. We then use a Microsprayer to deliver the tobramycin. All rats receive aerosolized treatment on the evening of Day 2, morning and evening of Day 3, and morning and evening of Day 4. On Day 5, we perform necropsies on all rats. The lungs and a sample of blood are collected at this time. Later during tissue processing, we collect the following information: lung compliance, inoculate lung lobe, non-inoculated lung lobe, liver, and spleen CFU/ml, and leukocyte counts.

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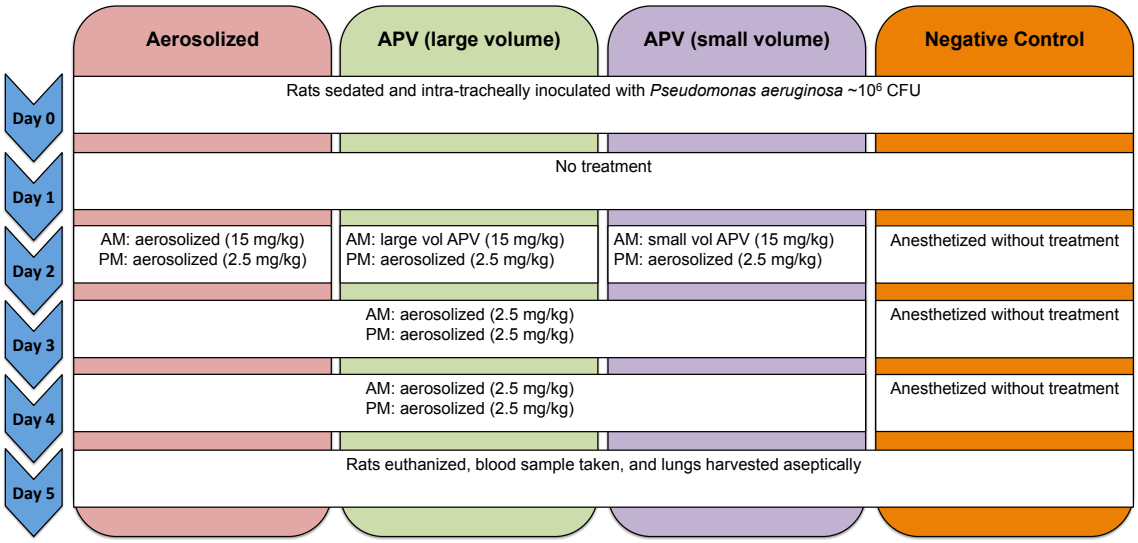

Figure 2: Treatment Schedule

2. We have started to run an *in vivo* experiment in hopes of observing the biofilm growth of *P. aeruginosa*. In this study, we grow the bacteria on a 96-well plate for 24 hours in order to imitate the biofilm growth that occurs in the body. When bacteria arrange themselves in this way, they are much more likely to withstand treatment with antibiotics. We then expose these biofilms to pre-oxygenated emulsion, unoxygenated emulsion, pre-oxygenated PFC, unoxygenated PFC, and sterile growth medium. The pre-oxygenated media receive continual oxygen support during exposure. After two hours we remove the biofilms from these emulsions and quantitatively measure the optical density of the biofilms in fresh growth medium using a spectrophotometer. The biofilms incubate for another six hours and then the second reading is taken. We measure the differences between the optical densities and interpret that as the amount of bacterial growth, and therefore a sign of how

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effective each of our exposures were. As seen in Figure 3, the emulsions were much more effective in killing the biofilms. There was no statistical significance between the unoxygenated and pre-oxygenated groups.

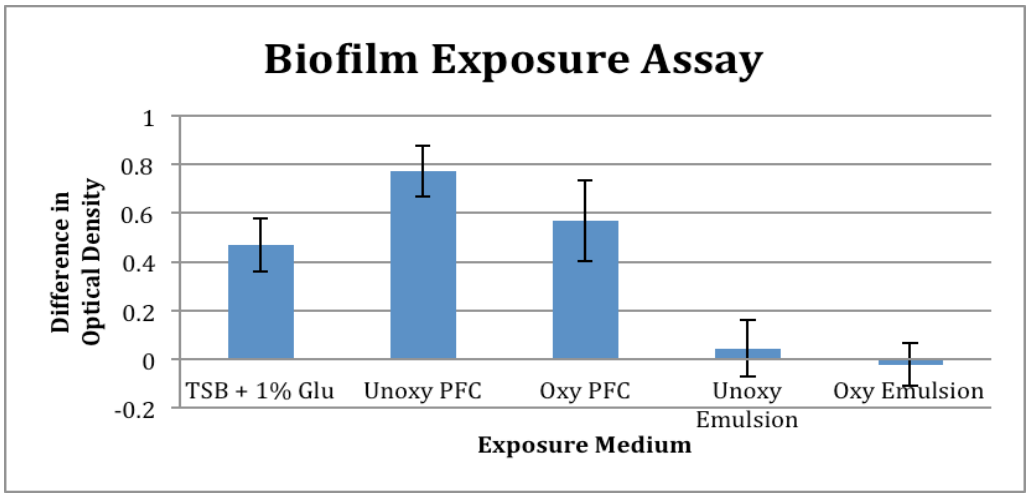

Figure 3: Emulsion Exposure Study Results.

IV. Timeline

By the end of the summer, we expected to have gathered enough data from our current rat trials to be able to move on to a larger model. We hoped to prove that APV is safe and effective in our small models and graduate to a sheep model. This will allow us to observe how APV works in a large mammal model, which is more similar to that of human anatomy. By the beginning of summer we had already defined our ideal concentration of tobramycin in our emulsion and begun collecting procedure data on rats. We continued this and while we have not moved on to large mammals yet, we will move on to sheep as soon as possible. To move to sheep requires having to calculate all new tidal and instillation volumes, anesthesia dosages, create new surgical equipment, perform preliminary sheep experiments, etc.. We are also now planning on moving to rabbits as a stepping stone to sheep, which requires its own separate set of calculations and preparations. Now that the

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laboratory has moved to Carnegie Mellon, that is the main focus of the next steps of the project.

VI. Results

The following graphs display the results from our experiment with APV. In Figure 4, the small volume APV treatment clearly does the best to alleviate the bacterial load in the lungs by the end of the study. Aerosolized only does slightly better than the control group, which is left untreated throughout the trial. We have run into slightly more confusing results in the large volume APV groups. As you can see in Figure 5, the APV group does only slightly better than the control, and the aerosolized is shown to be the most effective treatment. We believe this may be due to the increased volume load of PFC, which has been shown to promote the growth of bacteria in our *in vivo* experiments. Due to this factor, we've determined that small volume APV is the more effective and clinically promising treatment.

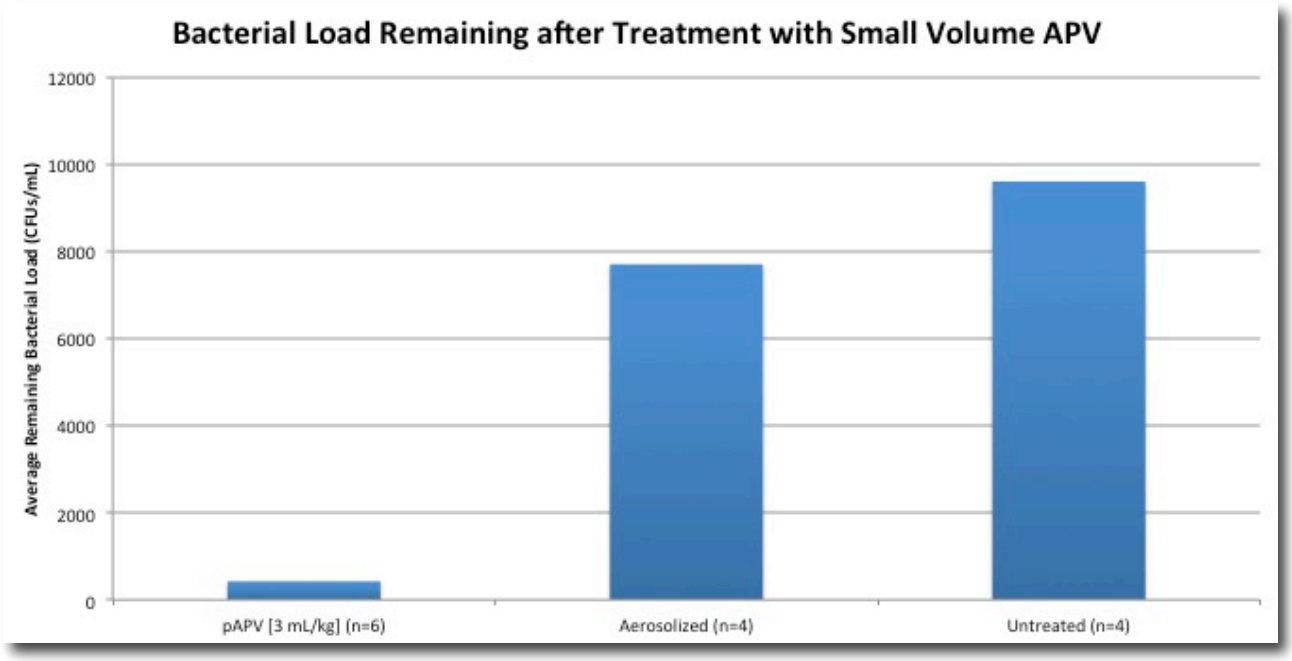

Figure 4: Small Volume APV Results.

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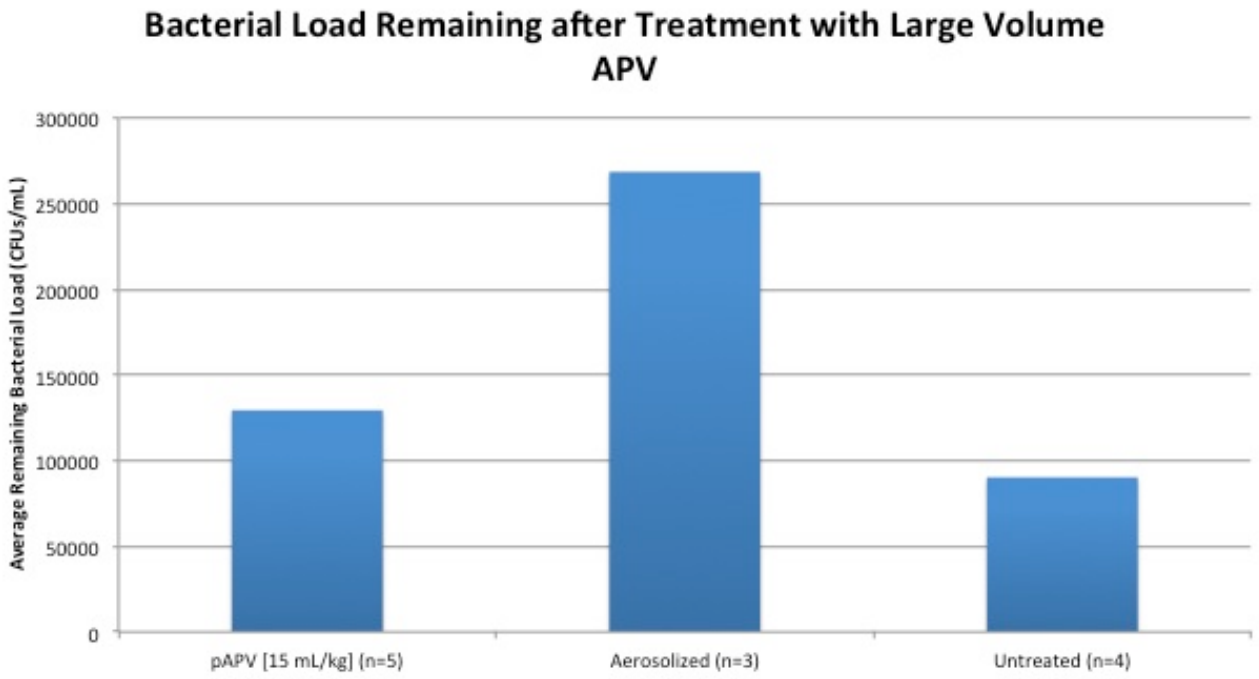

Figure 5: Large Volume APV Results.

VII. Conclusions

I. **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

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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

II. **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

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