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# Characterization of a *Pseudomonas aeruginosa* Cystic Fibrosis Clinical Isolate in Respiratory Infection Models

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Infection with *Pseudomonas aeruginosa* (*P.a.*) is a major cause of morbidity and mortality in Cystic Fibrosis (CF) patients. There is a clear need for new therapeutics against *P.a.* and rodent lung infection models are an important tool for understanding infection and therapeutic intervention in CF. However, the model response is strain specific based on factors such as phenotype and virulence. Here we assess the potential of a *P.a.* CF clinical isolate to induce infection in an acute mouse model and in addition to induce infection and inflammation in a rat model with the subsequent effects of tobramycin, an antibiotic routinely used in the treatment of CF, investigated.

Neutropenia in female BALB/c mice (17-22g, n=5 per group) was induced with cyclophosphamide because for some bacterial strains neutropenia is required to induce a robust infection (150mg/kg, day -4 and 100mg/kg day -1 prior to infection). Mice were intranasally infected with $1.15x10^6$ colony forming units (cfu)/ml *P.a.*. To assess antibiotic efficacy and routes of administration, the clinically used drug tobramycin (APP Pharmaceuticals) was used with vehicle (PBS) as a control. Mice were treated subcutaneously (1mg/kg and 16mg/kg) or intranasally (1mg/kg and 0.25mg/kg) BID and were euthanized 24 hours post infection by $CO_2$ asphyxiation, the lungs aseptically removed and homogenized to determine cfu/lung. Additionally, the *P.a.* isolate was embedded into agar beads, and male Wistar (Han) rats (190-240g, n=6-8/group) were intratracheally infected under isoflurane anaesthesia with either sham beads or $4.7x10^5$cfu/rat. They were treated with 50 mg/kg BID subcutaneous tobramycin, or vehicle. Cfu/lung and broncheoalveolar lavage fluid (BALF) cells were determined 2 days post-infection. Data are presented as mean ± SEM. Statistical analysis was performed using a Mann Whitney t-test or a Kruskall-Wallis one-way ANOVA with a Dunn's multiple comparison test as appropriate and $P<0.05$ was taken to be significant.

In the mouse Tobramycin treatment reduced bacterial load in a dose-dependent manner after both subcutaneous and intranasal doses (**Table 1**)

| **Route of Administration** | **Dose (mg/kg)** | **Log Bacterial Load** |
|---|---|---|
| Vehicle | N/A | 8.47±0.61 |
| s.c. | 1 | 7.51±0.67 |
| s.c. | 16 | 1.91±0.28 ** |
| i.n. | 0.25 | 3.98±0.57** |

| | | |
|---|---|---|
| i.n. | 1 | 1.83±0.33 |

**Table 1**: Lung bacterial load post tobramycin s.c. or i.n. administration. Data are presented as mean ± SEM. **, P<0.01 versus vehicle.

In the rat tobramycin also significantly reduced bacterial load in the lung (3.7±0.6 log cfu versus 6.1±0.3 log cfu, P<0.01, n=6). Infection significantly increased BALF neutrophils compared to sham ($7.61\pm1.37x10^6$ versus $0.11\pm0.02x10^6$ cells, P<0.001, n=7-8), which in turn was significantly reduced by tobramycin treatment ($2.88\pm0.58x10^6$ cells, P<0.01).

The clinical *P.a.* isolate caused infections in both mouse and rat lung models that could be treated with tobramycin,. Tobramycin was also shown to reduce BALF neutrophils in the rat model. Such studies with clinical isolates are important in the assessment of novel therapeutics and may help provide a better understanding of the diversity of pathogens present, their ability to cause infection and how they can be treated.