Mouse Model for Testing Therapeutics Against Pulmonary *Pseudomonas* Infection

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Development of a Mouse Model for *P. aeruginosa* Infection

1. Noninvasive instillation of bacteria to establish infection
2. Compare immunocompetent vs. immunocompromised models
3. Identify nonsubjective biometric endpoints
4. Multiple parameters to monitor therapeutic efficacy

References:
Intubation-Mediated Intratracheal (IMIT) Instillation

Considerations for inoculation:

1. Intranasal:
   • Upper respiratory track involvement
   • Variability in inoculum reaching lungs

2. Conventional intratracheal
   • Surgery can be technically difficult, slow
   • Potential for blood contamination

Model for IMIT

1. Intubate with catheter (Otoscope)
2. Insert blunt needle into catheter
3. Instill bacteria (50 µl + 100 µl air)
4. Less than 1 min per mouse

Video found at: www.jove.com/video/52261
Intubation-Mediated Intratracheal (IMIT) Instillation

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**Instillation of dye**

1. Broad distribution

**Instillation of Pa**

1. ~98% efficient delivery of inoculum
2. Reproducible over multiple animals

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Leukopenia Decreases LD$_{50}$ of *P. aeruginosa*

Balb/C Mice LD$_{50} = 10^{7.44}$ CFU

Leukopenic Balb/C Mice LD$_{50} = 10^{4.56}$ CFU

LD$_{50}$ in leukopenic mice decreased ~760-fold

Because of complications with antibiotic treatment of mice infected with high doses of bacteria, the leukopenic model was chosen as better suited for therapeutic testing (Lawrenz et al. Pathog Dis)

MDR clinical isolate UNC-D; Leukopenia induced by cyclophosphamide treatment (Lawrenz, M. B., et al. (2015). Pathog Dis.)
Leukopenia Decreases LD$_{50}$ of *P. aeruginosa*

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Establishing Biometric Endpoints

Leukopenic Balb/C Mice LD$_{50}$ = 10$^{4.56}$ CFU

Endpoint Check List:
1. Temperature ≤26.6°C
2. Heart rate ≤300 bpm
3. Oxygen levels ≤70%
4. Animal has lost its righting reflex

Temperature During Infection

MDR clinical isolate UNC-D; Leukopenia induced by cyclophosphamide treatment (Lawrenz, M. B., et al. (2015). Pathog Dis.)
Parameters That Can Measured in the Model

Survival/Mean Time to Death

Temperature During Infection

Bacterial Burden - Lungs

Dissemination - Spleen

Pathology – Lungs

Using the Model for Preclinical Testing of Therapeutics

Antibiotics – Polymyxin B vs. Meropenem

- **Polymyxin B**
  - EC$_{50}$ = 0.8291 µg/ml
  - MIC = 1 µg/ml (sensitive)

- **Meropenem**
  - EC$_{50}$ = 4.159 µg/ml
  - MIC = 8 µg/ml (intermediate)

MDR clinical isolate UNC-D; Unpublished data.
Using the Model for Preclinical Testing of Therapeutics

Antibiotics – Polymyxin B vs. Meropenem

- Dose dependent inhibition of bacterial numbers in the lungs
- At LOD in higher doses

MDR clinical isolate UNC-D. Therapy began at 3 h post-infection and continued q8 for 5 days. Continued monitoring for 7 days.
Using the Model for Preclinical Testing of Therapeutics

Antibiotics – Polymyxin B vs. Meropenem

- No change in survival
- No change in dissemination (data not shown)
- No change in pathology (data not shown)

- Dose dependent increase in MTD
- $ED_{50} = 1,258.5 \pm 73$ mg/kg/day
- Significant decrease in dissemination (data not shown)
- Significant decrease in pathology (data not shown)

MDR clinical isolate UNC-D. Therapy began at 3 h post-infection and continued q8 for 5 days. Continued monitoring for 7 days.
Using the Model for Preclinical Testing of Therapeutics

Testing novel therapeutics

This model has also proven amenable to:

• Different routes of administration of therapeutics, including but not limited to, subcutaneous and IP injection, intranasal and IMIT instillation, and aerosol delivery.
• Preclinical screening of both monotherapies and combination/adjunct therapies.

To date we have used this model to test 11 different therapeutics, which have included novel antibiotics, small compounds, and biologicals.