A standardized murine pneumonia model to evaluate antibiotic treatments

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Preclinical in vivo pharmacokinetic and pharmacodynamic models play a crucial role in assessing antimicrobial efficacy and provide the basis for the selection of dosing regimens in clinical applications. Differences in the methodology used to conduct preclinical in vivo models are extensive, thus limiting the results' comparability and reproducibility and possibly impeding successful translation to the clinic. To facilitate bench-to-bedside translation, and to accelerate and support the development of new antibiotics, it is advantageous to establish reliable and globally harmonized preclinical in vivo models.

The murine lung infection model is commonly used in proof-of-concept studies as well as PK/PD evaluation of antimicrobials against the major Gram-negative AMR pathogens *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii*. With the goal of developing a standard protocol for this model, experimental variables that may have a significant impact on the results were identified, as detailed in a complementary poster by Arrazuria *et al.* An expert workshop, "Advancing towards a standardized murine model to evaluate treatments for AMR lung infections", was held to discuss and explore the conduct and interpretation of these mouse lung infection models and the impact of each of the experimental variables.

Parameters with potential impact

Animals
- Mouse strain
- Sex
- Age
- Number animals/group
- Others (vendor, acclimatization, etc.)

Inoculum
- Source of bacterial strain
- Culture media
- Growth stage
- Inoculum preparation

Infection procedure
- Immunosuppression
- Anesthesia
- Infection route
- Infection volume
- Inoculum concentration

Panel discussion

Treatment and endpoint
- Time to start of treatment
- Baseline CFU
- Bacterial growth in mice
- Length of study
- Primary endpoint
  - Sample processing methods

Recommendations for standard parameters

Animals
- CD1 (outbred) mice
- Female mice
- Minimum of 3 d of acclimatization
- 6-8 weeks old animals
- 5-6 animals per group

Inoculum
- Include one *in vivo* validated strain from an accessible strain bank
- Use bacteria in logarithmic phase of growth

Standard murine pneumonia model

Infection procedure
- Use neutropenic mice
- Cyclophosphamide 150 mg/kg at day -4 and 100 mg/kg at day -1
- Intranasal infection route
- Inoculum of 50 µl

Treatment and end point
- Treatment at 2 hour post infection
- Baseline CFU of 6-7 log10
- Min. growth of 1 log10 CFU
- Length of study 26 h post infection
- Endpoint readout CFU/lung

A survey at the end of the workshop confirmed a consensus in favour of these recommendations among the participants. **Future perspective:** A standard murine lung infection protocol using these recommended parameters has been developed and is being validated with *P. aeruginosa, K. pneumoniae* and *A. baumannii* for the purpose of characterizing PK/PD of small molecule antibiotics in pre-clinical development.

Good practice recommendations

Animals
- Use animals of the same sex consistently in the same study. After preliminary study consider testing the effect in the other gender.
- Use animals from the same vendor.
- Adjust the number of animals to the power analysis if necessary.
- A minimum of acclimatization period is required.
- Animal randomization is encouraged.

Inoculum
- Time between inoculum preparation and its use in vivo should be short.
- Ensure inoculum viability and growth consistency in the whole experiment.

Infection procedure
- Anesthesia should be deep enough to allow the inoculum to settle in the lungs.
- Intra tracheal route should be considered for less pathogenic strains.
- If a lower inoculum is required, 20 µl is the recommended minimum.

Treatment and end point
- If longer experimental endpoint (26 h) are needed for additional outputs (3-4 d), take several time points including 26 h.
- Blinding the CFU counts if possible.

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