Neutropenic Guinea Pig Thigh Infection Model

Introduction

With antibiotic resistance on the rise, there is an urgent global need for discovery of new antibiotics. Determination of the pharmacokinetic-pharmacodynamic (PK/PD) properties of potential new antibiotics is vital to early antibiotic drug discovery and development. Animal models of infection have been instrumental in preclinical assessment of PK/PD and *in vivo* antimicrobial effectiveness of new antibiotics and help guide parameters used for clinical studies.

The rodent thigh infection model was first described by Selbie and Simon in 1952 and has since been modified and employed to examine the PK/PD and *in vivo* efficacy of antibacterial agents in different rodent species for multiple indications such as: pneumonia, skin and soft tissue infection, septicemia and intra-abdominal infections. The most commonly used thigh infection model is the neutropenic mouse thigh infection model, in which the mice are rendered immune-incompetent by treatment with cyclophosphamide.

Applications

- Determination of PK/PD indices related to efficacy (and prevention of resistance) of an antimicrobial agent.
- Determination of the time course of antimicrobial activity and post-antibiotic effect – concentration or time dependent; presence/absence of persistent effects.
- Identification of factors that affect the magnitude of PK/PD indices – CFU changes (short course therapy) vs. survival (long course therapy).
- Determination of dose, dosing interval and in vivo susceptibility break points.
- Testing/benchmarking antimicrobial drugs; determination of therapeutic equivalence of generic products.

The neutropenic mouse thigh-infection models have been extensively used in pre-clinical antibiotic development for determination of PK/PD and *in vivo* antimicrobial efficacy due to their inexpensive and reproducible nature. However, species-specific differences in drug adsorption and metabolism can vary depending upon the nature of the test article and delivery platform. These differences can lead to errors in the translation of interspecies pre-clinical PK/PD and efficacy data to the clinical setting, making selection of a relevant model an essential step in the progression of drug development.

At Noble, we have established a neutropenic guinea pig thigh infection model which can be used to determine the PK/PD and efficacy of antimicrobial agents against pathogenic bacteria. The guinea pig model has an advantage over the mouse model enabling the ability to analyze multiple blood samples from a single animal during the course of the study using a surgically implanted catheter. The model can also have clinical-translational advantages over that of the mouse or rat models in situations where the adsorption or metabolic pathways of the guinea pig more closely mimic that of the human.

This model can be used as a first-line test system for determination of PK/PD and *in vivo* efficacy, or as a complement to our already established mouse neutropenic thigh-infection model.

Protocol

In order to eliminate confounding effects of the immune system on antimicrobial activity, female guinea pigs were rendered neutropenic by IP administration of cyclophosphamide over the course of five days.

The neutropenic guinea pigs were randomly assigned into groups of six each and administered different inoculum doses of *Escherichia coli* ATCC 25922 or *Klebsiella pneumonia* ATCC 43816 ($1x10^5$ CFU, $1x10^7$

CFU, or 1x10^9 CFU) by IM injection into the left thigh to determine an optimal infectious dose. Three animals from each group were humanely sacrificed at 2-hours or 24-hours post-inoculation, and their thighs were aseptically removed, weighed and homogenized in sterile PBS. Serial dilutions of homogenates were prepared and plated onto Mueller-Hinton agar plates. Bacterial colonies were enumerated for each plate following incubation at 37°C for 24 hours.

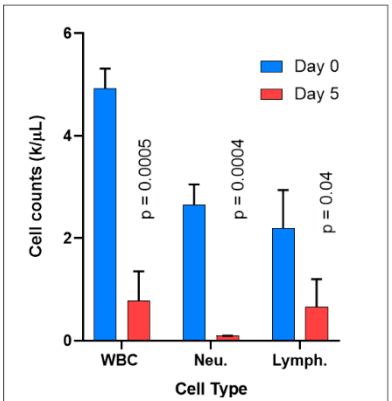

Figure 1. Blood cell counts in guinea pigs rendered neutropenic with cyclophosphamide treatment.

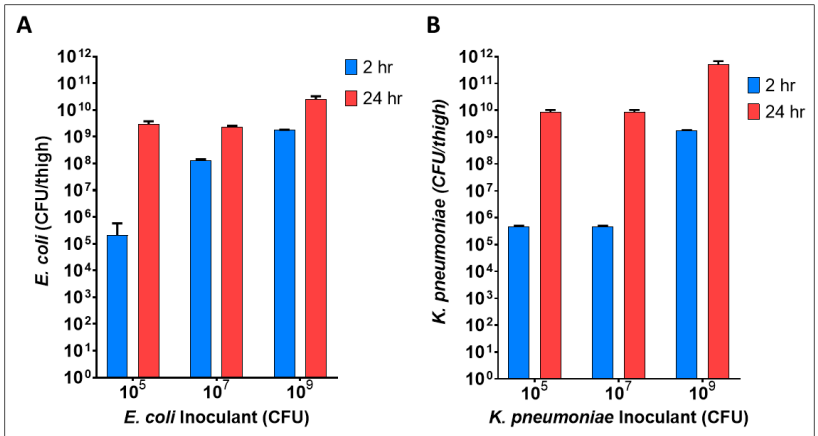

Figure 2. Titration of *E. coli* (A) and *K. pneumoniae* (B) in neutropenic guinea pigs.

To demonstrate the application of this model in testing the antibacterial therapeutic efficacy, neutropenic guinea pigs infected with *E. coli* were treated with Meropenem-a broad spectrum antibiotic used to treat a variety of bacterial infections. Female guinea pigs rendered neutropenic by administration of cyclophosphamide over a five day were challenge with 1 x 10^5 CFU *E. coli* ATCC 25922 by IM injection into the left thigh. Two hours following bacterial challenge, animals were administered 100 mg/kg meropenem or PBS by intraperitoneal injection. Thighs were aseptically removed 24 hours following bacterial challenge, weighed and homogenized in sterile PBS. Serial dilutions of homogenates were prepared and plated onto Mueller-Hinton agar plates. Bacterial colonies were enumerated for each plate following incubation at 37°C for 24 hours.

Results

Blood cell counts performed prior to induction of neutropenia and one day following the final dose of cyclophosphamide demonstrated that treatment significantly reduced total white blood cell and neutrophils and effectively induced neutropenia and lymphocytopenia (Figure 1).

There was an overall dose-dependent increase in the bacterial loads from thighs harvested at 2 and 24 hours post-infection in both *E. coli* and *K. pneumonia*e infection models (Figure 2). Peak bacterial loads at the 24 h time points ranged from 2.9 x 10^9 CFU/thigh to 2.5 x 10^{10} CFU/thigh for *E. coli* and 8.5 x 10^9 CFU/thigh to 5.3 x 10^{11} CFU/thigh for *K. pneumoniae*. There was a greater than 4-$\log_{10}$ increase in microbial load at 24 h compared to the 2 h time point with the 10^5 CFU inoculum for both bacterial strains demonstrating significant bacterial replication in the neutropenic animals over the 24 h period.

The model was tested in neutropenic guinea pigs infected with 1 x 10^5 CFU *E. coli* and treated with 100 mg/kg Meropenem. Results demonstrated a significant 2.9 $\log_{10}$ decrease in microbial load in animals treated with 100 mg/kg meropenem (Figure 3) showing the antibacterial efficacy of Meropenem.

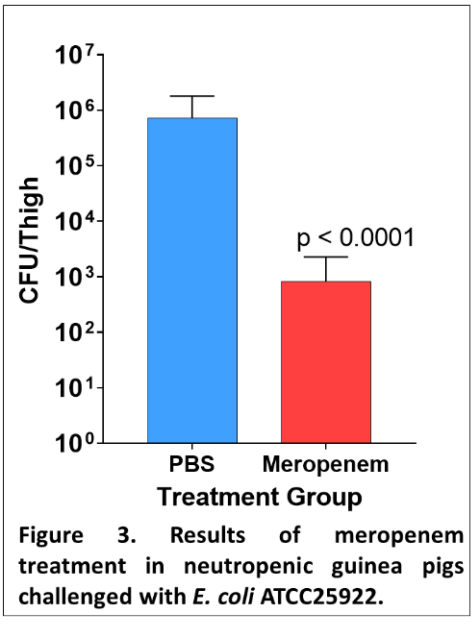

Figure 3. Results of meropenem treatment in neutropenic guinea pigs challenged with *E. coli* ATCC25922.

Summary

Rodent models of infection are essential tools for preclinical assessment of pharmacokinetic-pharmacodynamic and *in vivo* effectiveness of new antibiotics. Data resulting from their use guides parameters used for clinical studies and can aid in the establishment of antimicrobial breakpoints. The results herein show the establishment of reproducible and relevant bacterial thigh infection models of *E. coli* and *K. pneumonia* in neutropenic guinea pigs. Studies using the model may be performed with guinea pigs having a surgically-implanted catheter allowing for the analysis of multiple specimens from a single animal over the course of treatment, thus requiring fewer animals than protracted PK/PD studies in mice. The model can also have clinical-translational advantages over that of the mouse or rat models in situations in which the adsorption or metabolic pathways of the guinea pig more closely mimic that of the human, as has been observed with drugs that are substrates for catalysis by aldehyde oxidase (AOX)[1] and suggested for studies involving carbapenems (meropenem) as the guinea pig renal dehydropeptidase I (DHP-I), which hydrolyzes meropenem, is more similar to human than that of the mouse DHP-1 [2].

This model can be used as a first-line test system for determination of PK/PD and *in vivo* efficacy both as prophylactic and/or therapeutic, or as a complement to our already established mouse neutropenic thigh-infection model.

References

1. Crouch, R.D. et. Al. Xenobiotica. 2018 Mar; 48(3): 219–231.

2.Agudelo, M. et. al. Antimicrob Agents Chemother. 2014 Feb; 58(2): 1005–1018.