Neutropenic Mouse Thigh Model of Infection

Introduction

Complicated skin and soft tissue infections are frequently encountered in clinical practice and are a significant cause of morbidity and mortality in hospitalized patients. The neutropenic mouse thigh model of infection has been used extensively to test and benchmark antimicrobial drugs leading to a significant impact on our current knowledge of antimicrobial pharmacology. This model allows the quantitative comparison of different agents and different dosing regimes and the determination of the time-course of antimicrobial activity under conditions optimal for efficacy, i.e., neutropenia. Because the pharmacology of antimicrobial agents is affected by the presence of neutrophils, animals are commonly rendered neutropenic with cytotoxic agents like cyclophosphamide, the most frequently used immunosuppressive agent in animal infection models.

Noble Life Sciences, in collaboration with ImQuest BioSciences, has successfully developed a neutropenic mouse thigh model and demonstrated its use in a study to evaluate vancomycin for the treatment of a *Staphylococcus aureus* infection. The model will serve as a platform for the evaluation of the efficacy of novel anti-microbial compounds in the treatment of microbial infections.

Applications

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

Protocol

Twenty-one 5 to 6 week old female ICR (CD1) mice were made neutropenic by administration of two doses of cyclophosphamide (150 mg/kg and 100 mg/kg per mouse on days 1 and 4, respectively).

On Day 5, 18 mice were challenged with 0.1 mL of a 10^7 CFU/mL of *Staphylococcus aureus* 29213 (provided by ImQuest BioSciences) by intramuscular injection in the right thigh. One group of 3 mice (Group 2) was sacrificed 2 hours post inoculation and the other mice in groups of 3 mice each were treated with vancomycin (Groups 4-7) or vehicle control (Groups 1 and 3) at 2, 8, and 14 hours post-inoculation.

At 24-hours post inoculation (Groups 1, 2-7) or 2-hours post inoculation (Group 2), mice were sacrificed and the right thigh aseptically removed, weighed, and placed in a tube on ice for transport to ImQuest BioSciences for evaluation. Thighs were transferred to 15 mL polypropylene tubes and homogenized in 3 mL of sterile PBS with a hand held IKA works T25 generator with sterile 7 mm x 110 mm disposable rotor stator probe.

Serial ten-fold dilutions of the thigh homogenates were prepared in sterile PBS and 0.1 mL of four dilutions for each thigh was plated onto trypticase soy agar plates containing 5% sheep's blood, in duplicate. Bacterial colonies were enumerated for each plate following ~20 hours of incubation at 37° C.

Results

Results of colony counts are presented in Fig 1. Vancomycin effectively inhibited the replication of *S. aureus* resulting in a 4.4 (100 mg/kg) to 5.2 (800 mg/kg) $\log_{10}$ reduction in CFU/gram in the treated animals (Groups 4-7) compared to the untreated animals (Group 3).

No colonies were observed on plates for undiluted thigh homogenates from Group 1 animals. Back calculation of colonies from the Group 2 animals indicated that the starting inoculum was 1.3 x 10^6 CFU/mL.

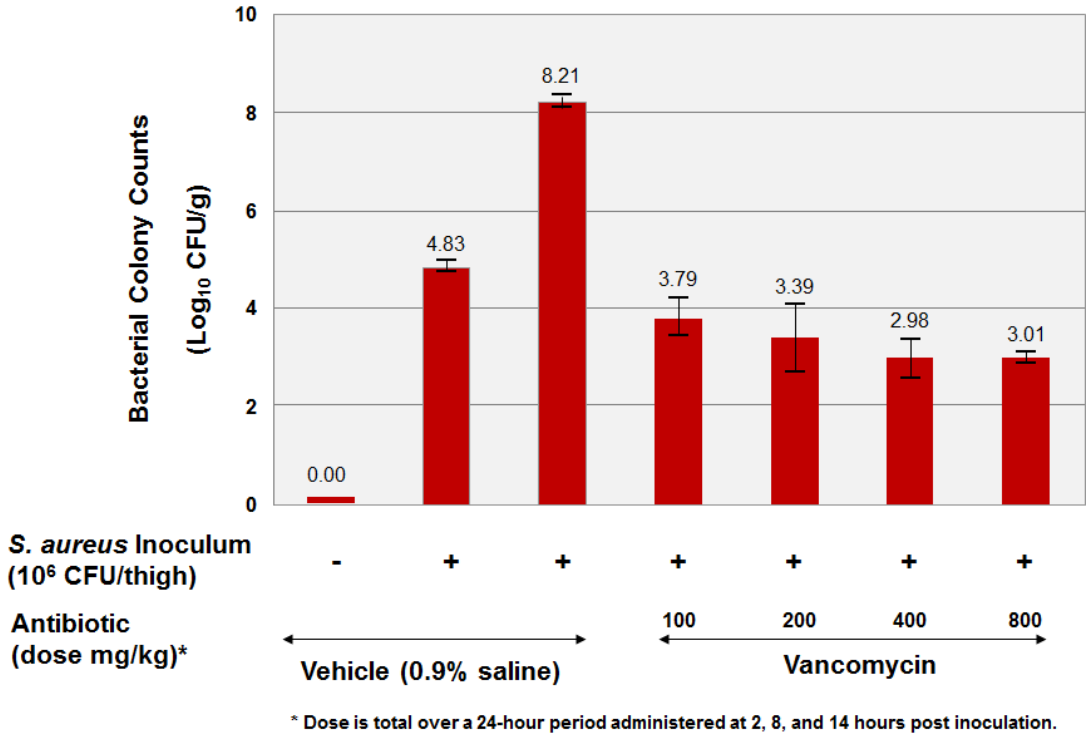

Figure 1 - Results of vancomycin treatment in neutropenic mice challenged with *Staphylococcus aureus* (Groups 2-7); group 1 was not inoculated. Following the bacterial challenge, mice were treated with either vehicle or vancomycin. The mice were sacrificed 24-hr post inoculation (Groups 1, 3-7) or 2-hr post inoculation (Group2); the total CFU/gram of thigh was measured for each group (n=3).

Summary

The results reported herein derived from a collaborative effort combining the animal model and drug development expertise of Noble Life Sciences (Gaithersburg, MD) with the microbiology expertise of ImQuest BioSciences (Frederick, MD).

The demonstrated success of this collaborative effort will allow for routine performance of animal models of infection, including the neutropenic thigh model, for the evaluation of new anti-microbial agents.