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Efficacy of Imipenem and Tigecycline in a Mouse Pneumonia Model Infected with *Acinetobacter baumannii*

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M. PULSE*, P. NGUYEN, J. PIERCE, P. RENICK, W. J. WEISS, J. SIMECKA
UNT Health Science Center - Preclinical Services, Ft. Worth, TX

*Contact Information:
UNT Health Science Center
3500 Camp Bowie Blvd
Fort Worth, TX 76107
www.hsc.unt.edu/preclinical
mark.pulse@unthsc.edu

Abstract

Background: *A. baumannii* (ABAU) is an important clinical pathogen primarily associated with nosocomial pneumonia and/or bacteremia infections. Due to a continued need for evaluating novel agents that treat drug-resistant ABAU infections, a mouse pneumonia model was developed that can evaluate antibiotic efficacy with both CFU (colony-forming unit) and survival endpoints.
Methods: Female CD-1 mice were rendered neutropenic by dosing Cytoxan (150/100 mg/kg at days -4/-1 pre-infection). Anesthetized animals were intranasally inoculated with one of two ABAU clinical isolates at 5.1 – 6.9 log_{10} CFU. For CFU studies, single antibiotic doses were administered intraperitoneally (i.p.) 4 hours after infection. Animals were euthanized 24 hours after antibiotic administration, and lungs were harvested and plated for CFU recovery. For survival studies, antibiotics were i.p. dosed 4 hours after infection, and continued twice daily for 72 hours. Survival rates were monitored for 10 days following infection.
Results: For CFU studies, lung counts for ABAU strain UNT091-1 increased from 6.82 log_{10} CFU at 4 hours post-infection to 8.22 log_{10} CFU at 28 hours, while lung counts for strain UNT092-1 increased from 7.53 log_{10} to 8.55 log_{10} CFU after 28 hours. Single doses of Imipenem (IMI) resulted in mean log_{10} CFU reductions ranging from 1.14 to 3.41 as compared to the untreated 28-hour controls for both strains, while single doses of Tigecycline (TIG) reduced mean log_{10} CFU counts by 0.51 to 2.31 for both strains. In the survival studies, animals infected with either ABAU strain showed 60 – 100% survival after 3.5 days of dosing IMI at 12.5 to 30 mg/kg, while 20 – 100% survived when dosed with TIG at 12.5 to 50 mg/kg.
Conclusions: Dose responses for both IMI and TIG were observed for both CFU and survival endpoints, and ABAU strain-dependent susceptibility was also observed in this disease model. These results suggest that this model can be useful for evaluating new antibiotics to treat ABAU pneumonia infections.

Introduction

Acinetobacter baumannii infections have become a major concern among healthcare facilities worldwide, which is primarily the result of two important facts. The first fact is that both the prevalence and incidence of antibiotic-resistance among *A. baumannii* clinical isolates have significantly increased over the last two decades. The second fact is that *A. baumannii* can survive for prolonged periods within the hospital-environment, thus increasing the likely hood of infectious spread among vulnerable patients. Additionally, *A. baumannii* can easily colonize mechanical ventilators and other devices, which is one of the reasons why hospital-acquired pneumonia (HAP) is the most common infection caused by *A. baumannii*.
With all of this in mind, there is an apparent need for new and improved antibiotics for the treatment of hospital-acquired *A. baumannii* infections. Once new drug candidates are identified, a portion of their pre-clinical evaluation should include identifying their possible use in relevant clinical indications such as *A. baumannii* HAP. To meet the need for non-clinical models that can be used to evaluate antibiotics against *A. baumannii* infections, we have established an experimental mouse model of acute pneumonia infection with clinical isolates of *A. baumannii*. Here we describe the use of this model to evaluate the efficacy of Tigecycline and Imipenem against two *A. baumannii* clinical isolates.

Methods and Materials

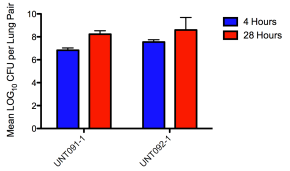
Minimum inhibitory concentrations (MICs): MICs were determined for Imipenem and Tigecycline against both *A. baumannii* clinical strains (UNT091-1 and UNT092-1) by using the microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI).
Mouse pneumonia model
Animals: Female, 5 - 6 wk old CD-1 mice (18 - 22 gm) were used according to the protocol approved by the UNTHSC-Institutional Animal Care and Use Committee.
Neutropenia: Mice were rendered neutropenic by intraperitoneally (i.p.) injecting cyclophosphamide at 150 mg/kg 4 days prior to infection, followed by 100 mg/kg on the day before infection.
Inoculum preparation & infection: *A. baumannii* strains (UNT091-1 and UNT092-1) were cultured overnight on tryptic soy agar (TSA), and then suspended to the desired inoculum size for each strain. Anesthetized mice (ketamine at 40 mg/kg, xylazine at 6 mg/kg) were infected with the prepared inocula by intranasal instillation.
Treatment: All doses of each antibiotic (Imipenem or Tigecycline) were administered by i.p. injection. For colony-forming unit (CFU) studies, mice were given a single dose of the designated antibiotic and specified dose amount (mg/kg) at 4 hours post-infection. For survival studies, antibiotics were initially administered 4 hours post-infection, and then dosed twice daily for 3 consecutive days.
Endpoints: For CFU studies, mice were euthanized at 4 (controls) and 28 hrs post-infection, their lungs were removed, homogenized, and then plated onto tryptic soy agar (TSA) that contained activated charcoal. For survival studies, mice were monitored for survival over 10 total days, which included a period of 7 days following the last antibiotic dose.

Panel 1: MIC Values for Imipenem and Tigecycline against *A. baumannii* Strains UNT091-1 and UNT092-1

	UNT091-1	UNT092-1
Imipenem	8 µg/mL	2 µg/mL
Tigecycline	2 µg/mL	2 µg/mL

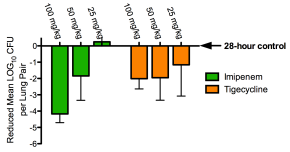
- MIC values determined by the microdilution method according to CLSI guidelines.
- *A. baumannii* reference strain **ATCC**19606 was included as a quality control for each MIC test (data not shown).

Panel 2: Lung CFUs of UNT091-1 and UNT092-1 at 4 and 28 Hours Post-Infection



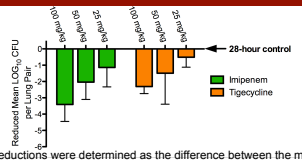
- CFU counts of lungs harvested at 4 & 28 hours post-infection for each strain.
- Lungs were homogenized, serial diluted, and plated onto TSA+charcoal.
- Error bars represent the standard deviation (SD) of the mean for each time point. (*n*=3)

Panel 3: Mean Log_{10} CFU Reduction of UNT091-1 in the Lungs of Antibiotic Treated Mice vs. 28-Hour Controls



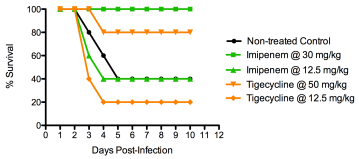
- Mean log_{10} CFU reductions were determined as the difference between the mean CFU counts of untreated 28-hour controls vs. antibiotic treated groups.
- Indicated doses (x-axis) represent the amount intraperitoneally administered as a single dose 4-hours after infection. Lungs were taken 28 hours after the final dose.
- Error bars represent the SD of the reduced mean CFUs for each group. (*n*=3)

Panel 4: Mean Log_{10} CFU Reduction of UNT092-1 in the Lungs of Antibiotic Treated Mice vs. 28-Hour Controls



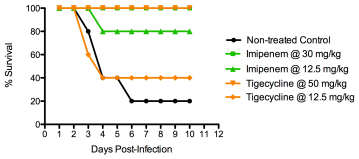
- Mean log_{10} CFU reductions were determined as the difference between the mean CFU counts of untreated 28-hour controls vs. antibiotic treated groups.
- Indicated doses (x-axis) represent the amount intraperitoneally administered as a single dose 4-hours after infection. Lungs were taken 28 hours after the final dose.
- Error bars represent the SD of the reduced mean CFUs for each group. (*n*=3)

Panel 5: % Survival of Mice having Lethal Pneumonia Infections with UNT091-1



- Percent survival of untreated and antibiotic treated mice for 10 days following infection with UNT091-1. (*n*=5)
- Indicated doses (legend) represent the amount intraperitoneally administered as a single dose 4-hours after infection, and then twice daily for 3 consecutive days.

Panel 6: % Survival of Mice having Lethal Pneumonia Infections with UNT092-1



- Percent survival of untreated and antibiotic treated mice for 10 days following infection with UNT092-1. (*n*=5)
- Indicated doses (legend) represent the amount intraperitoneally administered as a single dose 4-hours after infection, and then twice daily for 3 consecutive days.

Summary and Conclusions

- Minimum inhibitory concentration (MIC) values indicate that both *A. baumannii* clinical strains, UNT091-1 and UNT092-1, were equally sensitive to Tigecycline (Panel 1). However, the MIC value for Imipenem against UNT091-1 was 4-fold higher than the one generated for UNT092-1, suggesting that doses of Imipenem may be less efficacious in mice with respiratory infections caused by UNT091-1.
- Lung-associated CFUs for strain UNT091-1 increased from 6.82 log_{10} at 4 hours post-infection to 8.22 log_{10} at 28 hours, while the CFUs for strain UNT092-1 increased from 7.53 to 8.55 log_{10} over the same 28-hour period (Panel 2). The increase in lung CFUs over 28 hours for both strains indicated that a metabolically active and growing infection was generated in mice, which implied that this model could be used to evaluate the efficacy of antibiotics against *A. baumannii* respiratory infections.
- (Panels 3 & 4) While single doses of Imipenem at 50 and 100 mg/kg decreased lung CFUs to between 1.8 to 4.2 log_{10} in UNT091-1 infected animals, a single dose of Imipenem at 25 mg/kg did not effectively reduce total lung counts as compared to 28-hour controls. For mice infected with UNT092-1, single doses of Imipenem at 25, 50, and 100 mg/kg reduced total lung CFUs to between 1.1 to 3.4 log_{10} when compared to 28-hour controls. Single doses of Tigecycline at 25, 50, and 100 mg/kg effectively reduced total lung counts for both strains that ranged from 0.5 to 2.3 log_{10} CFU as compared to the total lung counts of 28-hour controls.
- (Panels 5 & 6) Multiple doses of Imipenem over 3.5 days at 30 mg/kg resulted in complete protection (100% survival) of mice that were lethally infected with either *A. baumannii* strain. Imipenem dosed at 12.5 mg/kg over 3.5 days resulted in the 40% survival of mice infected with UNT091-1, while the same doses of Imipenem (12.5 mg/kg for 3.5 days) resulted in an 80% survival of mice infected with UNT092-1. Tigecycline doses of 12.5 and 50 mg/kg over 3.5 days resulted in 20% to 80% of the mice surviving a lethal respiratory infection caused by UNT091-1. The same doses of Tigecycline in UNT092-1 infected mice resulted in 40% to 100% of the animals surviving over the duration of the study.
- These results suggest that this mouse model can be used in the pre-clinical evaluation of antibiotics (current or new) for the treatment of *A. baumannii* respiratory infections.

References

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