Efficacy of Topical and Systemic Antibiotics in a Mouse Superficial Skin Infection Model with *Staphylococcus aureus*

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Abstract

Background: Due to the continued need to thoroughly evaluate new antimicrobials for skin-associated infections, a mouse superficial skin infection (MSSi) model was developed in our lab to determine the efficacy of antibiotics against *Staphylococcus aureus* skin-associated infections. Here, we describe the evaluation of both topical and systemic antibiotics in this disease model of skin infection.
Methods: Anesthetized, female CD-1 mice were shaven and mechanically abraded within their sub-scapular regions. After a slightly reddened area was generated, 5.0 log10 CFU (colony-forming units) of *S. aureus* was inoculated onto the abraded site. Treatments were initiated 4 hours post-inoculation with either topical or systemic antibiotics, and treatment was continued twice daily for 3 additional days. Abraded skin sections were excised, washed, homogenized, and plated for CFU recovery 18 to 20 hours after the final treatment. Mean CFU counts of treated and untreated control groups were compared to determine antibiotics efficacy within this model.
Results: The CFU counts of abraded skin sections from untreated control groups increased from 3.4 log10 CFU at 4 hours post-inoculation to 6.3 log10 CFU after 4 days. When compared to 4-day untreated controls, the skin sections from animals topically treated with Bactroban (mupirocin) yielded mean log10 CFU reductions greater than 4.0, while intraperitoneal (i.p.) doses of Vancomycin at 25, 75, 150 mg/kg reduced mean log10 CFU counts by 0.9, 1.0, and 2.5, respectively. Tigecycline i.p. treatments at 5, 25, and 50 mg/kg reduced the log10 CFU skin-section counts by 1.3, 2.0, and 2.3, respectively, while i.p. doses of Linezolid at 10 at 20 mg/kg produced mean log10 CFU reductions that were less than 0.5.
Conclusions: Not only did this model clearly differentiate the efficacy of each antibiotic tested, but it also produced observable dose responses for Vancomycin and Tigecycline. These results demonstrate that the MSSi model can be useful in evaluating and comparing both topical and systemic antibiotics for skin-associated infections.

Introduction

Staphylococcus aureus is an important bacterial pathogen capable of causing various types of diseases in humans and other animals. Skin-associated pathologies, like folliculitis, cellulitis, and impetigo, are the most common types of diseases caused by *S. aureus*, and therapeutic options that are available for treating skin and soft-tissue clinical infections (SSTIs) have been significantly impacted due to the increased prevalence of methicillin-resistant *S. aureus* (MRSA) associated with SSTIs (1). Furthermore, infections caused by community-acquired MRSA (CA-MRSA) have become an epidemic within the US where the USA 300 strain is the most common cause of community-acquired, staphylococcal SSTIs (2, 4). Like hospital-acquired MRSA (HA-MRSA), CA-MRSA can be resistant to multiple antibiotics. However, CA-MRSA is considered to be more pathogenic than HA-MRSA, which is thought to be the result of a coordinated expression of several virulence factors that includes a cytotoxin identified as the Panton-Valentine leukocidin (PVL) factor (3). The issues of multi-drug resistance and enhanced virulence, combined with the current epidemic situation within the US, point to the fact that new and/or improved therapeutic options are needed to treat CA-MRSA infections.
To aid in the evaluation of therapeutics that treat staphylococcal SSTIs, a mouse superficial skin infection model (MSSi) was established by our lab that is capable of evaluating the efficacy of topical and systemic antibiotics against *S. aureus* skin infections. Here we describe the evaluation of a topical and three systemic antibiotics against a methicillin-susceptible *S. aureus* (MSSA) strain and a CA-MRSA strain (USA300).

Methods and Materials

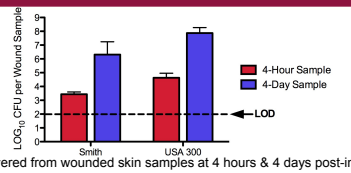
Bacteria: Smith was the MSSA strain and NRS688 (USA300; PVL+) was the CA-MRSA strain used in the MSSi studies. Overnight (>18 hours) plate cultures of each strain were used to generate the inocula for MSSi studies and minimum inhibitory concentration (MIC) assays.
Minimum inhibitory concentrations (MICs): MICs were determined for Vancomycin, Tigecycline, and Linezolid (Zyvox) against the MSSA and CA-MRSA strains using the microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI). Briefly, the three antibiotics were two-fold serially diluted in cation-adjusted Mueller-Hinton broth (CAMHB), and 5.0 log10 colony-forming units (CFU) for each strain was added to each drug-containing well of a 96-well plate. After overnight (>18 hours) incubation at 37°C, breakpoints were identified for each antibiotic, and strain susceptibility was determined according to CLSI guidelines. The ATCC reference strain of *S. aureus* (25923) was included as the quality control for each MIC test.
MSSi model: While under gas anesthesia (Isoflurane), the lower dorsal areas of 5 to 6 week old, female CD-1 mice was shaven and then disinfected with betadine/ethanol. A 7" disposable emery board (100/100 grit) was rubbed within the disinfected region until a slight reddened and glistening wound appeared. A 5 µL volume from a 7.0 log10 CFU/mL inoculum prepared for each strain was separately applied onto the abraded area of each mouse. Antibiotic treatment was initiated 4 hours following infection, and was continued twice daily for 3 consecutive days. Animals were gas euthanized 18 to 20 hours after the final antibiotic dose, and the skin abrasions were harvested, washed, homogenized and plated for CFU recovered.

Panel 1: MICs of Vancomycin, Tigecycline, Linezolid against MSSA & CA-MRSA strains

	MSSA (Smith)	CA-MRSA (USA300)
Vancomycin	1 µg/mL	0.5 µg/mL
Tigecycline	0.25 µg/mL	0.25 µg/mL
Linezolid (Zyvox)	2 µg/mL	2 µg/mL

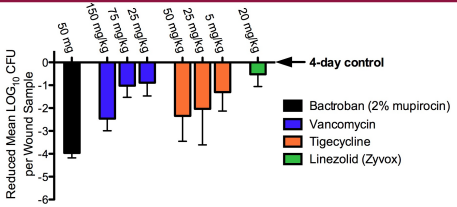
- MIC values determined by the microdilution method according to CLSI guidelines.
- ATCC, *S. aureus* reference strain 25923 was included as a quality control for each MIC test (data not shown).

Panel 2: Growth of MSSA (Smith) & CA-MRSA (USA300) in the MSSi model



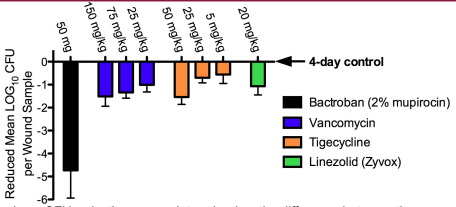
- CFU counts recovered from wounded skin samples at 4 hours & 4 days post-infection.
- Skin homogenates were plated onto selective (mannitol salt) & non-selective media.
- Limit of detection (LOD) was 2.0 $\log_{10}$ CFU, & error bars represent the standard deviation (SD) of the mean for each time point. (*n*=5)

Panel 3: Antibiotic Efficacy in the MSSi model against MSSA (Smith)



- Mean $\log_{10}$ CFU reductions were determined as the difference between the mean CFU counts of untreated 4-day controls vs. antibiotic treated groups.
- Indicated doses (x-axis) represent the total amounts administered initially at 4 hours post-infection, & then continued twice daily for 3 consecutive days.
- Bactroban was topically applied, & the other antibiotics were intraperitoneally dosed.
- Error bars represent the SD of the reduced mean CFUs for each group. (*n*=5)

Panel 4: Antibiotic Efficacy in the MSSi model against CA-MRSA (USA300)



- Mean $\log_{10}$ CFU reductions were determined as the difference between the mean CFU counts of untreated 4-day controls vs. antibiotic treated groups.
- Indicated doses (x-axis) represent the total amounts administered initially at 4 hours post-infection, & then continued twice daily for 3 consecutive days.
- Bactroban was topically applied, & the other antibiotics were intraperitoneally dosed.
- Error bars represent the SD of the mean reduced CFUs for each group. (*n*=5)

Panel 5: Treated & Untreated, Mean $\log_{10}$ CFU Wound Counts for Smith (MSSA) & USA300 (CA-MRSA)

	4-HR CONTROL	4-DAY CONTROL	BACTROBAN (2% mupirocin)	VANCOMYCIN (mg/kg)			TIGECYCLINE (mg/kg)			LINEZOLID (ZYVOX)
				150	75	25	50	25	5	20 mg/kg
SMITH (MEAN ± SD)	3.4 ± 0.16	6.3 ± 0.93	2.4 ± 0.22	3.9 ± 0.53	5.3 ± 0.51	5.4 ± 0.58	3.9 ± 1.11	4.3 ± 1.58	5.0 ± 0.83	5.8 ± 0.54
USA300 (MEAN ± SD)	4.6 ± 0.33	7.9 ± 0.4	3.2 ± 1.21	6.4 ± 0.43	6.5 ± 0.25	6.9 ± 0.31	6.3 ± 0.32	7.2 ± 0.22	7.3 ± 0.39	6.8 ± 0.38

Summary and Conclusions

- Minimum inhibitory concentration (MICs) values for Vancomycin, Tigecycline, and Linezolid (Panel 1) indicate that both the Smith (methicillin-sensitive *S. aureus*; MSSA) and USA300 (community-acquired, methicillin resistant *S. aureus*; CA-MRSA) strains were sensitive to all 3 antibiotics. These antibiotics are FDA approved agents for treating skin and soft tissue infections (SSTIs) caused by staphylococci (4), which is why they were selected for evaluation in the mouse superficial skin infection (MSSi) model. Additionally, Bactroban (2% mupirocin) served as the positive control in the MSSi studies.
- The wound-associated CFU for the MSSA strain (Smith) increased from 3.4 log10 at 4 hours to 6.3 log10 at 4 days post-infection, and the CFU for the CA-MRSA strain (USA300) increased from 4.6 log10 at 4 hours to 7.9 log10 at 4 days. These results suggest that both *S. aureus* stains are capable of producing a stable skin-associated wound infection for an extended period of time (4 days) in this model.
- For the Smith strain, mean 4-day CFU associated with skin wounds decreased by: 3.9 log10 with 7 applications of Bactroban cream; 0.9 to 2.4 log10 with 7 i.p. doses of Vancomycin at 25 -150 mg/kg; 1.3 to 2.4 log10 with 7 i.p. doses of Tigecycline at 5 – 50 mg/kg; 0.5 log10 with 7 i.p. doses of Linezolid (Zyvox) at 20 mg/kg.
- Mean 4-day CFU for USA300 infected wounds decreased by: 4.7 log10 with 7 applications of Bactroban cream; 1.0 to 1.5 log10 with 7 i.p. doses of Vancomycin at 25 – 150 mg/kg; 0.6 to 1.6 log10 with 7 i.p. doses of Tigecycline at 5 – 50 mg/kg; 1.1 log10 with 7 i.p. doses of Linezolid (Zyvox) at 20 mg/kg.
- As compared to the Smith strain, the USA300 strain (NRS688) appears to have an enhanced ability to generate an infection with greater severity in this model, which may have negatively impacted the efficacy of Vancomycin and Tigecycline against the USA300 strain (Panels 2, 3, 4). This may be attributable to this strain being positive for the Panton-Valentine leukocidin (PVL) factor, which has been shown to play a key role in the progression of certain staphylococcal infectious diseases (4).
- Dose responses were limited in this model for both *S. aureus* strains. Additionally, strain-dependent responses to antibiotic treatment were observed in this model. These results suggest that the MSSi model can be a valuable tool for evaluating new and current agents (systemic or topical) for the treatment of staphylococcal skin and soft tissue infections (SSTIs).

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