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The Successful Treatment of Experimental Methicillin-resistant *Staphylococcus aureus* (MRSA) Keratitis with Topical Penicillin

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Abstract

Purpose: The systemic treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections with penicillin is contraindicated due to resistance. It is unknown if this resistance can be overcome by an excessive amount of drug delivered directly to the site of infection. This study determined whether penicillin, in a high concentration, can overcome resistance to treat MRSA keratitis in a rabbit model.

Methods: Both corneas of 32 rabbits were intrastromally infected with 2000 CFU of MRSA. The corneal epithelium was removed in the left eyes to mimic corneal ulceration while the epithelium remained in the right eyes. After 4h, 8 rabbits were euthanized to determine amounts of corneal bacteria at the onset of treatment. The remaining rabbits were divided into 3 groups (n=8): 1) 6% penicillin; 2) 2.5% vancomycin; 3) saline. Both eyes were treated topically every 15 minutes for 5h. One hour after treatment, the amounts of corneal bacteria were determined and analyzed for treatment effectiveness and bactericidal effect.

Results: Penicillin was more effective than vancomycin in reducing MRSA when the corneal epithelium was removed ($P \leq 0.05$), but was only as effective when the epithelium remained. Both treatments reduced colony counts compared to saline with and without epithelium ($P \leq 0.05$). Penicillin was bactericidal in eyes with and without epithelium while vancomycin was only bactericidal in eyes without epithelium.

Conclusion: We demonstrated that penicillin can overcome resistance to successfully treat MRSA keratitis in a rabbit model. Penicillin was as or more effective than 2.5% vancomycin, depending if the corneal epithelium was removed.

Keywords: Keratitis; Methicillin-resistant *Staphylococcus aureus*; Penicillin; *Staphylococcus aureus*

Introduction

By definition, a bacterial isolate designated as a methicillin-resistant *Staphylococcus aureus* (MRSA) indicates that the isolate is resistant to all β-lactam antibiotics including the penicillins, cephalosporins, and carbapenems [1]. The mechanism of resistance to methicillin is mediated via the *mec* operon, part of the Staphylococcal cassette chromosome *mec* (SCC*mec*) [2]. This element contains the *mecA* gene, which encodes for an altered penicillin-binding protein (PBP2a) that has a lower affinity for binding β-lactam antibiotics and therefore allows cell wall synthesis to proceed in their presence [2]. This eliminates the clinical use of β-lactam antibiotics, including penicillin the first β-lactam antibiotic, during systemic MRSA infections [2].

With regards to penicillin, resistance in *Staphylococcus aureus* infections was originally due to production of penicillinase, an enzyme that cleaves the β-lactam ring of penicillin and prevents the penicillin molecule from binding to the penicillin-binding proteins to inhibit cell wall biosynthesis. This led to the development of other β-lactam antibiotics that were unaffected by penicillinase, the first of which was methicillin.

The Clinical and Laboratory Standards Institute (CLSI) antibiotic breakpoints that determine whether bacteria are resistant to antibiotics are based on the concentrations of antibiotics achieved

in the serum after systemic administration. There are no antibiotic breakpoints to determine antibiotic resistance for topical ocular therapy. Topical antibiotic therapy is based on the assumption that antibiotic concentrations achieved in ocular tissue are equal to or greater than those achieved in the serum after systemic therapy.

The systemic resistance breakpoints for β-lactam antibiotics can be quite low. *Staphylococcus aureus* isolates with Minimal Inhibitory Concentrations (MICs) ≥0.25 and ≥4.0 µg/ml are considered resistant to penicillin and cefoxitin (an antibiotic used to determine methicillin-resistance) respectively [3].

Although MRSA isolates are considered resistant to β-lactam antibiotics, the isolates can still produce MICs that are susceptible to higher concentrations of these antibiotics. The gap in knowledge to be addressed by this study is to determine whether penicillin can reach high enough concentrations in the ocular tissue during topical therapy to be effective. If successful, penicillin could be used as a possible alternative therapy to treat MRSA ocular infections in patients without a penicillin allergy.

We have previously demonstrated that the topical fluoroquinolones gatifloxacin 0.3% and levofloxacin 1.5% can overcome resistance and successfully treat fluoroquinolone-resistant and methicillin-resistant *Staphylococcus aureus* corneal infections in a New Zealand White (NZW) rabbit model [4,5]. We have also demonstrated that penicillin can successfully treat experimental keratitis caused by multiple isolates of penicillin-resistant *Staphylococcus aureus* [6]. However, no previous studies have been completed demonstrating the successful treatment of experimental MRSA keratitis using topical penicillin. If successful, this study will demonstrate a "Proof of Principle" that penicillin, in a high concentration, can overcome resistance and successfully treat a MRSA corneal infection in a rabbit keratitis model.

Materials and Methods

Experimental Drugs

A solution of 6% penicillin (PEN) (100,000 units/ml [7]) was prepared in IV saline from a 5,000,000 units' vial of penicillin G potassium for Injection (Buffered Pfizerpen®, Pfizer, New York, NY). A solution of 2.5% (25 mg/ml [7]) vancomycin (VAN) (standard of care control) was prepared in IV saline from a 500 mg vial of Vancomycin HCl for Injection USP (Fresenius Kabi, Lake Zurich, IL). Aliquots of PEN and VAN were stored frozen at -20°C until use. On the day of the experiment, aliquots were thawed and used for dosing. IV saline (0.9% Sodium Chloride Injection USP [Baxter Healthcare Corp. Deerfield, IL]) served as the negative control (CON). Thirty-seven µl drops were instilled using a Rainin EDP electronic pipet set in the multi-dispense mode. The test drugs were kept on ice during dosing.

Bacterial Strain

A MRSA keratitis isolate (K950) was used in this study. The cefoxitin MIC was 64 µg/ml, therefore the isolate was reported as methicillin-resistant. The penicillin MIC was 16 µg/ml and the vancomycin MIC was 2.0 µg/ml.

Animals

Female NZW rabbits ranging from 1.1-1.4 kg in weight were obtained from Charles River's Oakwood rabbitry. University of Pittsburgh IACUC approval was obtained and institutional and federal guidelines regarding animal experimentation were followed.

Experimental Protocol

A total of 32 NZW rabbits were used in duplicate trials of 16 rabbits. Corneal epithelial defects (6.5 mm) were created in the left eyes using an Amoils epithelial scrubber (abraded epithelium), while the epithelium of the right corneas remained intact (intact epithelium) to determine if removal of the corneal epithelium affected drug efficacy by enhancing drug penetration and to mimic a corneal ulcer [8,9]. The corneas were intrastromally inoculated in both eyes with ~2000 colony forming units per eye (CFU/eye) of MRSA in 25 µl following systemic anesthesia with 40 mg/kg of ketamine and 4 mg/kg of xylazine and topical anesthesia with 0.5% proparacaine. After 4 hours, the rabbits were divided into the 4 treatment groups (n = 8): A) 6% Penicillin; B) 2.5% Vancomycin; C) Saline; and D) No Treatment (euthanized before treatment to determine baseline CFU in the corneas at the Onset of Therapy) (ONSET). Topical therapy consisting of one drop every 15 minutes for 5 hours (21 doses) was initiated. One hour after treatment was completed, the animals were euthanized and the corneas were harvested for CFU determination.

Statistical Analysis

The data from the two trials were combined. The CFU + 1 were Log_{10} converted. The CFU data were analyzed with True Epistat (Richardson, TX) using Kruskal-Wallis with Duncan's Multiple Comparisons. Significance was established at the $p \leq 0.05$ confidence level.

Bactericidal Effect

Bactericidal effect was defined as a 3-Log_{10} (99.9%) or greater decrease in combined median corneal colony counts between the test antibiotics and the ONSET.

Results

The results of the topical antibiotic therapy on eyes with abraded epithelium are presented in Figure 1. Eyes treated with 6%

penicillin [median Log_{10} (CFU+1)/ml = 0.0; range of colony counts = 0.0-2.1 Log_{10} (CFU+1)/ml] and 25 mg/ml vancomycin [median Log_{10} (CFU+1)/ml = 1.3; range of colony counts = 0.0-2.1 Log_{10} (CFU+1)/ml] had significantly fewer MRSA colony counts than eyes treated with the saline control [median Log_{10} (CFU+1)/ml = 3.9; range of colony counts = 1.3-6.0 Log_{10} (CFU+1)/ml] and the ONSET [median Log_{10} (CFU+1)/ml = 4.4; range of colony counts = 4.0-5.0 Log_{10} (CFU+1)/ml] (p = 0.05). There were no significant differences in colony counts when comparing eyes treated 6% penicillin and 2.5% vancomycin. Penicillin and vancomycin both produced a >3 Log_{10} decrease in colony counts compared to the ONSET. This indicates that penicillin and vancomycin were bactericidal in eyes with abraded epithelium.

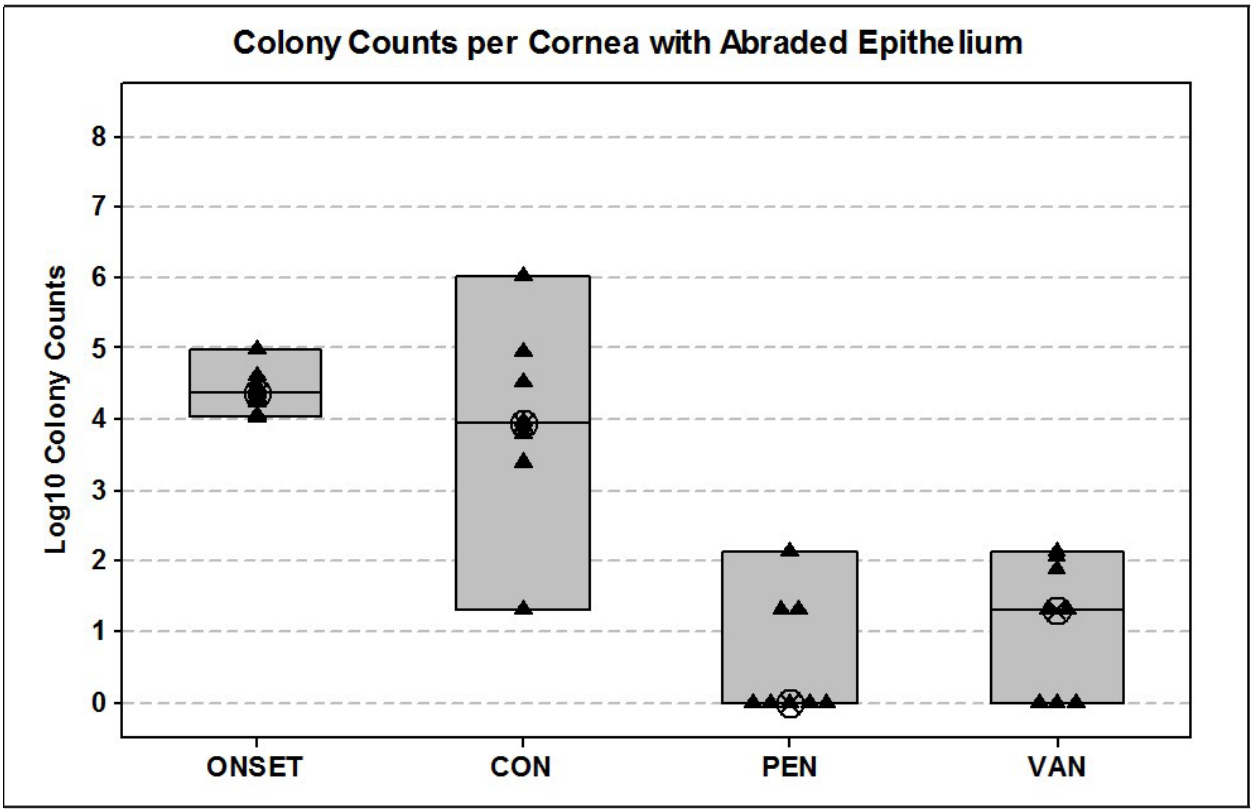

Figure 1: Demonstrates the median (line with ⊗) and range (gray box) of MRSA colony counts per cornea in corneas with abraded epithelium for each treatment group. Both PEN and VAN demonstrated significantly fewer colony counts compared with the ONSET and CON (PEN = VAN < ONSET = CON; p = 0.05). There were no significant differences between PEN and VAN and between CON and ONSET. The decreases in colony counts produced by PEN and VAN were bactericidal compared to ONSET. ▲ represent individual data points.

The results of the topical antibiotic therapy on eyes with intact epithelium are presented in Figure 2. Similarly, eyes treated with 6% penicillin [median Log_{10} (CFU+1)/ml = 0.0; range of colony counts = 0.0-2.0 Log_{10} (CFU+1)/ml] showed a significant decrease compared to both the saline control, [median Log_{10} (CFU+1)/ml = 7.0; range of colony counts = 6.3-7.9 Log_{10} (CFU+1)/ml] and ONSET [median Log_{10} (CFU+1)/ml = 4.8; range of colony counts = 3.4-5.4 Log_{10} (CFU+1)/ml] but the ONSET was significantly less than the saline control (p = 0.05). However, in eyes with intact epithelium, penicillin also demonstrated a significant difference when compared with 25 mg/ml vancomycin [median Log_{10} (CFU+1)/ml = 2.5; range of colony counts = 0.0-6.5 Log_{10} (CFU+1)/ml] (p=0.05). In eyes with intact epithelium, penicillin demonstrated a bactericidal effect compared to the ONSET whereas vancomycin did not. These results suggest that penicillin can penetrate the corneal epithelium just as well as, or even better than vancomycin.

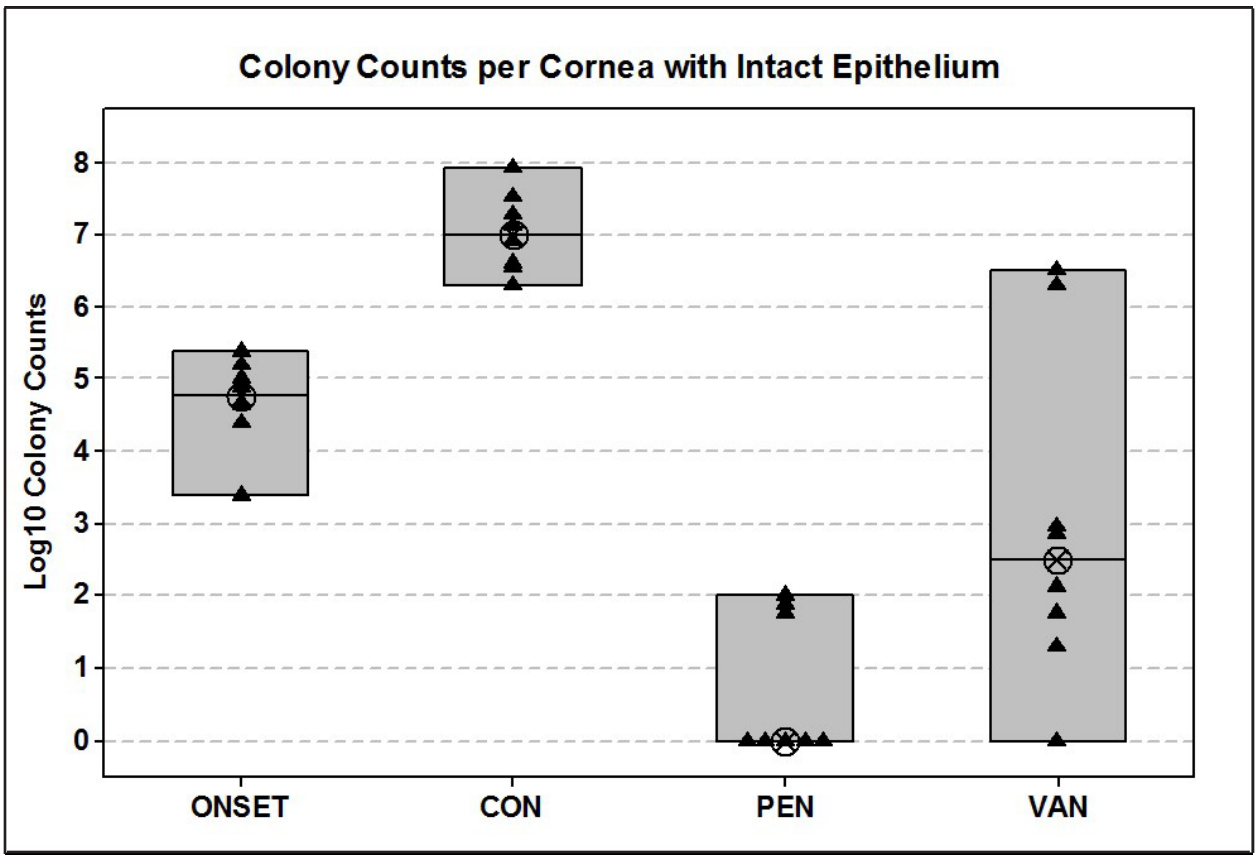

Figure 2: Demonstrates the median (line with ⊗) and range (gray box) of MRSA colony counts per cornea in corneas with intact epithelium for each treatment group. PEN significantly decreased corneal colony counts compared with VAN. Both demonstrated significantly fewer corneal colony counts compared with the ONSET and all were significantly less than CON (PEN < VAN < ONSET < CON; $p = 0.05$). The decrease in corneal colony counts produced by PEN was bactericidal compared to ONSET. ▲ represent individual data points

Discussion

This experiment was designed to demonstrate an important "proof of principle" that penicillin could be used to treat an experimental MRSA keratitis. Although these bacteria are considered resistant to β-lactams, the susceptibility standards used to determine resistance are based on systemic treatment rather than topical treatment. Currently there are no antibiotic breakpoints to determine susceptibility for topical treatment. Based on our previous studies, we believe that treating topically can deliver sufficiently high concentrations of antibiotic to the site of infection to overcome resistance and treat the infections, even in the case of a MRSA infection [4,5].

The efficacy of topical penicillin was demonstrated in comparison to topical vancomycin against MRSA isolate K950 (penicillin MIC 16 μg/ml). Penicillin demonstrated significant decreases of MRSA colony counts in comparison to vancomycin, saline control, and ONSET in eyes with intact corneal epithelium. However, in eyes with abraded corneal epithelium, penicillin produced significant decreases of MRSA colony counts in comparison to only the saline control and ONSET. Penicillin produced a bactericidal decrease in eyes with abraded and intact corneal epithelium while vancomycin was only bactericidal in eyes with abraded corneal epithelium. This suggests that penicillin, when administered topically, can achieve a concentration ≥16 μg/ml in the corneal stroma for a sufficient time to treat the infection. Since penicillin is a time-dependent antibiotic, maintaining this concentration at the site of infection for significant periods of time is critical. Further studies are warranted to determine the time that penicillin can remain at or above this concentration during topical treatment.

In the current study, the corneal epithelium does not appear to hinder the efficacy and penetration of penicillin. However, in another study by Kowalski et al, topical penicillin was tested against multiple *Staphylococcus aureus* isolates with elevated MICs to penicillin, and it was determined that the corneal epithelium acted as a barrier against the penetration of topical penicillin into the

corneal stroma [6]. This could be a result of the different isolates of *Staphylococcus aureus* used in the previous study having higher MICs to penicillin. Further testing is indicated to determine whether the corneal epithelium acts as a barrier to penicillin, and if so, to what degree it prevents the penicillin from penetration into the corneal stroma.

Additional studies are warranted to test other β-lactam antibiotics such as cefazolin, which is the standard fortified treatment of methicillin-susceptible *Staphylococcus aureus* keratitis, for the treatment of experimental MRSA keratitis. Determining the topical efficacy of these other β-lactam antibiotics against MRSA could prove to be very beneficial at a time in which antibiotic resistance is a major health concern.

In this study we demonstrated that penicillin could be used as an effective topical treatment for MRSA keratitis. By treating topically, we are able to show that penicillin is able to achieve sufficiently high concentrations in the corneal stroma to successfully treat a methicillin-resistant *Staphylococcus aureus* keratitis caused by an isolate deemed resistant to all β-lactam antibiotics. Without current development of many new antibiotics, this could prove to be a useful alternative treatment for MRSA keratitis.

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