The Effectiveness of an Improved Combination Therapy for Experimental *Staphylococcus aureus* Keratitis

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ABSTRACT

**Introduction:** Antibiotic and steroid combination therapies, such as tobramycin with dexamethasone, are often used in ophthalmology to treat or prevent infection and inflammation. The purpose of this study was to use a model of *Staphylococcus aureus* keratitis to quantify and compare the effectiveness of a standard tobramycin and dexamethasone combined therapy, with each drug individually, and with a new formulation of the two drugs in a xanthan gum vehicle.

**Methods:** Rabbit corneas were intrastromally injected with a methicillin-sensitive *S. aureus* (MSSA) or a methicillin-resistant *S. aureus* (MRSA) strain. Rabbit eyes were treated every hour from 10 to 15 hours postinfection (PI) with 0.1% dexamethasone, 0.3% tobramycin, 0.3% tobramycin with 0.1% dexamethasone, or 0.3% tobramycin with 0.05% dexamethasone in a xanthan gum vehicle (ST). Slit lamp examinations (SLE) were performed on infected eyes and pathology scored at 15 hours PI. At 16 hours PI, colony forming units (CFUs) per cornea were quantified.

**Results:** The CFUs in eyes treated with dexamethasone alone were similar to untreated control eyes for MSSA or MRSA infections. All other treatment groups had significantly less CFUs per cornea than untreated eyes. The eyes treated with the ST formulation had significantly fewer CFUs per cornea than all other treatment groups when infected with MSSA or MRSA. The SLE scores of MSSA or MRSA infected eyes treated with tobramycin alone were similar to untreated control eyes. All other treatment groups had significantly lower SLE scores than untreated controls eyes, but were not significantly different from each other.

**Conclusion:** The results of this study demonstrated that the tobramycin and dexamethasone combination therapy with a xanthan gum vehicle has an improved bactericidal effectiveness compared to the commercially available formulation, and maintains a similar anti-inflammatory effect while containing half the amount of steroid.

**Keywords:** antibiotic; dexamethasone; drug delivery; keratitis; *Staphylococcus aureus*; steroid; tobramycin; xanthan gum vehicle
INTRODUCTION

*Staphylococcus aureus* is a common cause of anterior eye infections that can be sufficiently severe to cause a significant loss in visual acuity.1,2 Because *S. aureus* is a potentially devastating problem for the anterior eye, drug formulations used to treat or prevent infections must be effective for this organism. Combined steroid and antibiotic therapy is used empirically for inflammation of the anterior eye when there is a risk of infection and for treating infections in this region.3 The combination therapy of tobramycin and dexamethasone has been widely used to treat common anterior eye infections (eg, red eye or blepharitis) and the inflammation associated with them. Although this combination has been analyzed experimentally in the anterior eye infected with *Pseudomonas* yielding positive results, no experimental in vivo studies have been published that quantify the effectiveness of the formulation in the *S. aureus*-infected anterior eye.4

Although the commercially available formulations of tobramycin with dexamethasone have a well-recognized clinical effectiveness, an improvement in terms of greater drug delivery has now been achieved by a new enhanced formulation. Like the standard formulation, the tobramycin and dexamethasone enhanced formulation (ST) contains 0.3% tobramycin, but has a reduced amount of dexamethasone (0.05%). The vehicle of the ST formulation is based on a new xanthan gum polymer suspension technology that is designed to enhance contact time and drug delivery.5 Xanthan gum is a water-soluble polysaccharide that has been found to have adherence properties that could make it useful in ocular medications.6 Xanthan gum as part of an ocular suspension was found to be well tolerated and comfortable.7 Furthermore, the ST formulation demonstrated longer settling times compared to the commercial formulation in vitro and increased bioavailability of both tobramycin and dexamethasone in rabbit eyes following topical administration.8

The present study was designed to determine the effectiveness of the ST formulation for treating *S. aureus* keratitis in the rabbit eye.

MATERIALS AND METHODS

Bacterial Strains and Infection

A well-characterized methicillin-sensitive *S. aureus* (MSSA), strain 8325-4, and an uncharacterized methicillin-resistant *S. aureus* (MRSA) keratitis isolate, strain 70490, were used in this study. Bacteria were grown in tryptic soy broth (TSB) overnight at 37°C. The overnight culture was inoculated into fresh TSB (1:100) and grown to log phase at 37°C. To infect rabbit corneas, log phase bacteria were serially diluted in TSB to 10,000 colony forming units (CFUs) per mL. Bacteria were diluted and plated on tryptic soy agar (TSA) to quantify the inoculum. Rabbit corneas were intrastromally injected with 100 bacteria in 10 µL of TSB.

Animals

Rabbits were anesthetized with 1:5 xylazine (100 mg/mL; AnaSed; Lloyd Laboratories, Shenandoah, IA, USA) and ketamine hydrochloride (100 mg/mL; KetaThesia; Butler Animal Health Supply, Dublin, OH, USA). Prior to infection, proparacaine hydrochloride (0.5 %; Bausch and Lomb, Tampa, FL, USA) was topically applied to all eyes. At the time of killing, all animals were anesthetized and administered a lethal overdose of pentobarbitol (Sigma-Aldrich, St Louis, MO, USA).