ORIGINAL ARTICLE

Effectiveness of a new tobramycin (0.3%) and dexamethasone (0.05%) formulation in the treatment of experimental Pseudomonas keratitis

Clare McCormicka, Armando Caballeroa, Aihua Tanga, Charles Balzlië, Jenny Songb and Richard O’Callaghan'a

a Department of Microbiology, University of Mississippi Medical Center, Jackson, MS, USA
b Division of Biostatistics, Health Cruiser Consulting, Plano, TX, USA

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ABSTRACT

Objective: To quantitatively determine, in a Pseudomonas keratitis model, the anti-inflammatory and bactericidal properties of a new formulation of tobramycin (0.3%) and dexamethasone (0.05%) that utilizes a xanthan gum vehicle.

Research methods: In a randomized and masked fashion, rabbit corneas (n ≥ 16 eyes per group) were intrastromally injected with 10³ colony-forming units (CFU) of P. aeruginosa. Eyes were untreated or were administered a single drop every 5 min between 16 and 17 h postinfection (PI) and then a single drop every 30 min between 17 and 22 h PI, a total of 15 drops of either 0.1% dexamethasone and 0.3% tobramycin (TobraDex®; Tdex) or a new formulation 0.3% tobramycin and 0.05% dexamethasone with xanthan gum (TobraDex ST; ST). Slit lamp examination scores (SLE ± SEM) were derived from grading seven parameters at 22 h PI. Rabbits were sacrificed at 23 h PI and the log CFU ± SEM per cornea was determined.

Results: Untreated eyes had SLE scores of 11.11 ± 0.43 and had log CFU of 7.27 ± 0.06. Eyes treated with Tdex, as compared to the untreated eyes, had significantly lower SLE scores (7.39 ± 0.21, p < 0.0001) and significantly fewer bacteria (6.32 ± 0.29 log CFU, p = 0.0213). Eyes treated with ST had a SLE score (6.56 ± 0.19) that was significantly lower than both the untreated eyes (p < 0.0001) and the eyes treated with Tdex (p = 0.0124). Furthermore, eyes treated with ST had significantly fewer log CFU (5.78 ± 0.30) than untreated eyes (p < 0.0001) or eyes treated with Tdex (p = 0.0434).

Conclusions: The ST formulation with xanthan gum demonstrated statistically superior anti-inflammatory and bactericidal properties as compared to Tdex.

Limitations: Variations in inoculation procedures produced limited eye-to-eye differences in the infection.
Introduction

Combined prophylactic antibiotic and steroid therapy is used empirically to treat the anterior eye for inflammation where there is the risk of infection. A formulation of tobramycin and dexamethasone (0.3% tobramycin and 0.1% dexamethasone; TobraDex* [Tdex]) is used to treat common eye infections (e.g., red eye or blepharoconjunctivitis) or when inflammatory processes compromise the eye1–6. Although this established formulation has well-recognized effectiveness, an improvement in terms of greater drug delivery has now been achieved in a new formulation.

The enhanced formulation (ST), like the standard formulation, contains 0.3% tobramycin but a reduced amount of dexamethasone (0.05%) in a vehicle featuring a new xanthan gum polymer suspension technology (TobraDex ST™). This vehicle is designed to enhance contact time and thus provide improved drug delivery7. Xanthan gum is a water-soluble polysaccharide that has been found to have adherence properties that could be useful in ocular medications8. Furthermore, the use of xanthan gum as part of an ocular suspension was found to be a well-tolerated and comfortable formulation9. The new formulation with xanthan gum, as compared to Tdex, demonstrated longer settling times in vitro and increased bioavailability of both tobramycin and dexamethasone in rabbit eyes following topical administration10. Although a quantitative human study of this design is not possible for ethical reasons, the present study, using a rabbit model of Pseudomonas keratitis, was designed to quantify the effectiveness of ST in reducing inflammation and in killing bacteria. Direct comparison of the new and established formulations is included. The results show that ST produced significantly reduced inflammation and superior bactericidal activity as compared to Tdex.

Methods

Bacteria

Pseudomonas aeruginosa strain 27853 was used in this study. It is a well-documented strain of P. aeruginosa for testing antibiotics11–13 and causes a well-characterized keratitis in rabbits13–15. A single colony of P. aeruginosa was grown in tryptic soy broth (TSB) overnight at 37°C. An aliquot (1:100) was inoculated into fresh TSB and grown to log phase at 37°C. Log phase bacteria were serially diluted in TSB to 100,000 colony-forming units (CFU) per ml. Bacteria were diluted and plated on tryptic soy agar (TSA) to quantify the inoculum.

Experimental keratitis

Specific pathogen-free, adult New Zealand White rabbits were used (n ≥ 16 corneas per group). These animals were housed according to the institutional guidelines and tenets of the Association of Research in Vision and Ophthalmology Resolution on the Use of Animals in Ophthalmic and Vision Research. Animals were anesthetized with 1:5 xylazine (100 mg/ml; Rompum; Miles Laboratories, Shawnee, KS) and ketamine hydrochloride (100 mg/ml; Ketaset; Fort Dodge Animal Health, Fort Dodge, IA). Prior to infection, proparacaine hydrochloride (0.5%; Bausch and Lomb, Tampa, FL) was topically applied to all eyes. Corneas of anesthetized rabbits were intrastromally injected with 10³ bacteria in 10μl of TSB. At the time of sacrifice, all animals were anesthetized and administered a lethal overdose of pentobarbitol (Sigma-Aldrich, St. Louis, MO).

Treatment regimen

In a randomized and masked fashion, at 16 h post-infection (PI), eyes were either untreated or a single topical drop of 0.3% tobramycin with 0.1% dexamethasone (Tdex), or 0.3% tobramycin with 0.05% dexamethasone in a xanthan gum vehicle (ST) was applied topically. Drops were administered every 15 min for the first hour of treatment (16–17 h PI). Starting at 17.5 h PI, drops were administered every 30 min until 22 h PI.

Slit lamp examination

At 22 h PI, slit lamp examination (SLE) of pathological changes in rabbit eyes was performed by two masked observers using a Topcon SL-7E biomicroscope (Koaku Kikai K.K., Tokyo, Japan)16–19. Each of seven parameters was graded on a scale ranging from 0 (none) to a maximum of 4 (severe): injection, chemosis, iritis, hypopyon, corneal infiltrate, fibrin in the anterior chamber, and corneal edema. The sum of these grades for an eye, after averaging, determined the SLE score. The SLE score could range from 0 (normal eye) to 220 (severely inflamed eye).
to a theoretical maximum of 28. A summary of the scoring system is in Table 1.

Colony-forming unit determination
At 23 h PI, rabbits were anesthetized, sacrificed, and corneas harvested to quantify CFU per cornea. Corneas were placed into sterile phosphate-buffered saline (0.1 M, pH 7.2; PBS) and homogenized. Corneal homogenates were serially diluted (1:10) in fresh PBS and plated, in triplicate, on TSA. Plates were incubated at 37°C overnight and the log CFU ± SEM per cornea were determined.

Statistical analysis
All statistical analyses were performed using statistical analysis software (SAS-PC version 9.1.2, SAS Institute, Cary, NC, USA). All data were analyzed by using the Kruskal–Wallis test to determine the significance of the overall comparison among the groups followed by the Wilcoxon rank sum test for pair-wise comparisons. CFU determination data of the treated groups were further compared. The percent of eyes with the log CFU above the mean control log CFU was analyzed using Fisher's exact test. All tests were two-sided with 95% confidence level; \( p < 0.05 \) was considered significant.

The power analysis for this study used a sample size calculation with 80% statistical power and 95% confidence level. A study with 22 subjects in each group would be sufficient to detect a difference of at least 1 in mean SLE score between the groups when the standard deviation (SD) is less than 0.8. The measured difference between the groups in this study was an average SLE score of 0.83 with a SD between 0.90 and 0.98. A power calculation indicated that the actual statistical power was 82% when a mid-point of 0.94 SD was used. Therefore, the present study exceeded the planned power of 80%.

Results
Rabbits were infected with Pseudomonas aeruginosa strain 27853 and at 16 h PI treatment began with a topical drop administered every 15 min during the first hour and then every half hour until 22 h PI (a total of 15 drops). At 22 h PI, the eyes were examined using a slit lamp and seven parameters of pathologic changes were analyzed. Rabbits were then sacrificed and the corneas were harvested for CFU determination.

At 22 h PI, eyes in the untreated eye group had an average SLE score of 11.11. In contrast, eyes treated with Tdex had an average SLE score of 3.72 less than the untreated control, a difference which was significant (\( p = 0.0213 \)) (Table 2; Figure 1). The eyes treated with ST had, at 22 h PI, an average SLE score of 4.55 lower than the untreated control, a difference which was statistically significant (\( p < 0.0001 \)). Also, the eyes treated with ST had an average SLE score of 0.83 less than eyes treated with Tdex (\( p = 0.0124 \)).

Rabbits were sacrificed at 23 h PI and the log CFU per cornea was determined. Untreated eyes had over 10 million bacteria per cornea (Table 3). Eyes treated with Tdex had 0.95 fewer log CFU per cornea as compared to the untreated control group (\( p = 0.0213 \); Figure 2). The eyes treated with ST had, at 22 h PI, an average SLE score of 4.55 lower than the untreated control, a difference which was statistically significant (\( p < 0.0001 \)). Furthermore, the eyes treated with ST, as compared to eyes treated with Tdex, had significantly fewer corneas with bacterial burdens above the mean control (0% vs. 31.25%; \( p = 0.0434 \)).

<table>
<thead>
<tr>
<th>Table 1. Scoring system for slit lamp examinations*</th>
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<tbody>
<tr>
<td>Anatomical site</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Conjunctiva</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cornea</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Anterior chamber</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Iris</td>
</tr>
</tbody>
</table>

*The slit lamp examination score (SLE) is the sum of the grades for the seven parameters. Scores of two or more masked observers are averaged to obtain a final SLE score for an eye at a given time point\(^{16-19}\).
Table 2. Comparison of SLE scores at 22h PI

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>Range</th>
<th>p-values*</th>
<th>Versus untreated control</th>
<th>Versus Tdex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>11.11</td>
<td>2.14</td>
<td>0.43</td>
<td>8.5–15.38</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tdex (0.3% tobramycin and 0.1% dexamethasone)</td>
<td>7.39</td>
<td>0.98</td>
<td>0.21</td>
<td>6–9.5</td>
<td>0.0213</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ST (0.3% tobramycin and 0.05% dexamethasone with xanthan gum)</td>
<td>6.56</td>
<td>0.90</td>
<td>0.19</td>
<td>5.38–8.5</td>
<td>&lt;0.0001</td>
<td>0.0124</td>
<td></td>
</tr>
</tbody>
</table>

*p-values of two-sided Wilcoxon rank sum test based on n-values of 25 for the untreated group and 22 for each treated group

Figure 1. Decreases from control values in SLE score following treatment with ST or Tdex. Seven parameters of inflammation were graded and the scores combined to determine the SLE score at 22h PI. Eyes treated with tobramycin (0.3%) and dexamethasone (0.1%) (Tdex) had reduced inflammation (i.e., lower SLE score) as compared to the untreated control. Eyes treated with tobramycin (0.3%) and dexamethasone (0.05%) with xanthan gum (ST) had significantly reduced SLE scores from both the untreated control eyes and from eyes treated with Tdex. *p = 0.0124 as compared to the Tdex treated eyes. (n = 22 per group; SLE score ± SEM; p ≤ 0.05 was considered significant)

Table 3. Comparison of log CFU per cornea at 23h PI

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>Range</th>
<th>p-values</th>
<th>Versus untreated control*</th>
<th>Versus Tdex†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>7.27</td>
<td>0.26</td>
<td>0.06</td>
<td>6.86–7.72</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tdex (0.3% tobramycin and 0.1% dexamethasone)</td>
<td>6.32</td>
<td>1.18</td>
<td>0.29</td>
<td>4.17–8.00</td>
<td>0.0213</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ST (0.3% tobramycin and 0.05% dexamethasone with xanthan gum)</td>
<td>5.78</td>
<td>1.20</td>
<td>0.30</td>
<td>3.88–7.23</td>
<td>0.0001</td>
<td>0.0434</td>
<td></td>
</tr>
</tbody>
</table>

*p-values of two-sided Wilcoxon rank sum test as compared to the untreated group based on n-values of 21 for the untreated group and 16 for the treated groups
†Fisher’s exact test was used to determine the statistical difference between the two treatment groups
Discussion

The development of the xanthan gum vehicle has provided a formulation that delivers tobramycin and dexamethasone more efficiently than the standard formulation\(^\text{10}\). The present study demonstrates that the improved drug delivery afforded by the xanthan gum based-vehicle results in significantly improved therapy of experimental *Pseudomonas* keratitis. The 0.3% tobramycin component of the new formulation provided substantial bacterial killing for an infection that, when usually treated with tobramycin, involves a fortified concentration of 1.33% or higher and with the inclusion of cefazolin for enhanced microbial killing\(^\text{20,21}\). The extensive bacterial killing afforded by the 0.3% tobramycin in the ST formulation reflects the enhanced drug delivery by the vehicle. This potent bacterial killing would be expected to have considerable value when used as an ocular medication. Likewise, the suppression of inflammation with the new formulation was significantly better than that achieved by the standard formulation despite the fact that the new formulation contains a dexamethasone concentration half that of the standard formulation. A vehicle with xanthan gum has been shown by others to provide greater drug delivery\(^\text{7}\). The findings herein demonstrate that the new technology does in fact result in an improvement in the therapy of infection. Scoper *et al.* have demonstrated that after topical application of the ST formulation, the concentration of both dexamethasone and tobramycin was greater in the rabbit tear fluid and ocular tissues as compared to the Tdex formulation\(^\text{10}\). They also determined the concentration of dexamethasone at 60 min after a single topical drop (45 \(\mu\)l) of the ST formulation was applied to rabbit eyes, and found that the concentration of dexamethasone in the cornea of rabbits was 2.8 \(\mu\)g/ml\(^\text{10}\). In a similar experiment using 0.1% dexamethasone acetate, Leibowitz *et al.* observed a concentration of 1.05 \(\mu\)g/ml in corneal tissue\(^\text{22}\). Leibowitz *et al.* used a concentration of dexamethasone that is double that found in the ST formulation and the applied drop was slightly larger (50 vs. 45 \(\mu\)l) as compared to the experiment performed by Scoper *et al.*\(^\text{22}\). However, the concentration of dexamethasone achieved by the ST formulation was more than double that of the 0.1% dexamethasone acetate formulation tested by Leibowitz *et al.*

The results presented herein show that the new tobramycin–dexamethasone formulation is superior to the standard formulation. This has important implications for a variety of anterior eye diseases. The study was conducted in a rabbit model of keratitis because it is a well-established model and models of other anterior eye diseases (e.g., blepharitis, conjunctivitis) are either not well-established or not reproducible. The findings imply that the new formulation will be of value in treating other anterior eye diseases, including

![Figure 2. Decreases from control values in log CFU per cornea following treatment with ST or Tdex. Rabbits were sacrificed at 23 h PI and the corneas were harvested and homogenized. Corneal homogenates were serially diluted and plated on TSA. Eyes treated with tobramycin (0.3%) and dexamethasone (0.1%) (Tdex) had a lower log CFU ± SEM per cornea than the untreated control eyes. However, eyes treated with the new formulation of tobramycin (0.3%) and dexamethasone (0.05%) with xanthan gum (ST) had fewer log CFU than both the untreated control eyes and the eyes treated with Tdex which was statistically significant. *p = 0.0434 between the two groups (n = 16 per group; p ≤ 0.05 was considered significant)*](image)
conjunctivitis, blepharokeratoconjunctivitis, and other inflammatory processes.

This experimental keratitis model exhibits a severe form of corneal pathology. The *Pseudomonas*-infected rabbit eye is analogous to the *Pseudomonas*-infected human eye in terms of intense corneal infiltrate, hypopyon formation, ulcer formation, and, if allowed to continue long enough, the perforation of the cornea. The limitations inherent in this study lie in the precision with which the individual infections are initiated; that is, the varying factor is the exact quantity of bacteria injected into each cornea. These injections are performed using microsyringes to achieve maximal precision. However, on retraction of the needle, a variable, yet small, amount of leakage of the inoculum can occur. Such leakage contributes directly, in an uncontrollable fashion, to the animal-to-animal variations. Another variation that can occur among animals is their immune response to the infection. Animals are standardized relative to size, age, and source, but there are still inherent (genetic) variations in these outbred animals.

An important consideration of any medical treatment is the risk of side-effects of the therapy. Corticosteroid use can cause increased ocular pressure, and prolonged administration has been associated with cataract formation. Another question surrounding the use of steroid therapy is whether it will adversely affect the effectiveness of antibiotic therapy. However, it has been shown that the action of antibiotics is not significantly inhibited by the concurrent administration of steroid therapy.

The more efficient drug delivery, coupled with the present evidence of increased effectiveness in suppressing inflammation and killing bacteria, indicate that ST will be valuable in multiple clinical settings and is expected to offer a useful alternative to Tdex for treating ocular conditions of the anterior eye.

**Conclusion**

When treating experimental *Pseudomonas aeruginosa* keratitis, the ST suspension demonstrates statistically superior anti-inflammatory and bactericidal properties as compared to the Tdex suspension.

**Acknowledgments**

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