Mycotic Antimicrobial Localized Injection

A Randomized Clinical Trial Evaluating Intrastromal Injection of Voriconazole

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Purpose: To determine if there is a benefit to adjuvant intrastromal voriconazole (ISV) injections for primary treatment of filamentous fungal keratitis.

Design: Outcome-masked, randomized controlled clinical trial.

Participants: Patients with moderate vision loss resulting from a smear-positive fungal ulcer.

Methods: Study eyes were randomized to topical natamycin plus ISV injection versus topical natamycin alone.

Main Outcome Measures: The primary outcome of the trial was microbiological cure on 3-day repeat culture analysis. Secondary outcomes included microbiological cure on 7-day repeat culture analysis; 3-week and 3-month best spectacle-corrected visual acuity; infiltrate or scar size or both; rate of perforation; therapeutic penetrating keratoplasty (TPK); and other adverse events.

Results: A total of 151 patients with smear-positive ulcers were screened and 70 were enrolled at Aravind Eye Hospital, Pondicherry, India. Baseline cultures grew Fusarium in 19 samples (27%), Aspergillus in 17 samples (24%), and other filamentous fungi in 19 samples (27%) and showed negative results in 13 samples (19%). Those randomized to ISV injection had 1.82 times the odds of 3-day culture positivity after controlling for baseline culture status (95% confidence interval [CI], 0.65–5.23; P = 0.26, bias-corrected logistic regression) and 1.98 times the odds of positive 7-day culture results, after controlling for baseline culture status (95% CI, 0.69–5.91; P = 0.20, bias-corrected logistic regression). Those randomized to ISV injection showed 0.5 logMAR lines (approximately 0.5 Snellen lines) of decreased visual acuity (95% CI, −2.6 to 3.6 lines; P = 0.75) and 0.55 mm worse infiltrate or scar size or both at 3 months after controlling for baseline values (95% CI, −0.13 to 1.25; P = 0.11). Intrastromal voriconazole injections showed a 2.85-fold increased hazard of perforation after controlling for baseline infiltrate depth (95% CI, 0.76–10.75; P = 0.12) but no difference in the rate of TPK (hazard ratio, 0.95; 95% CI, 0.44–2.04; P = 0.90).

Conclusions: There seems to be no benefit to adding ISV injections to topical natamycin in the primary treatment of moderate to severe filamentous fungal ulcers. Studies consistently suggest that voriconazole has a limited role in the treatment of filamentous fungal ulcers. Ophthalmology 2019;126:1084-1089 © 2019 by the American Academy of Ophthalmology

Supplemental material available at www.aaojournal.org.

Fungal corneal ulcers, because of their poor outcomes and the lack of evidence to guide treatment, present a therapeutic challenge to clinicians.1 In the tropics, fungal infection can account for upward of 50% of corneal ulcers.1–3 In the United States, fungal keratitis infections range from 35% of corneal ulcers in South Florida to 4% in temperate climates such as Los Angeles.4 The Mycotic Ulcer Treatment Trials (MUTT) I and II (MUTT II) were 2 National Eye Institute-funded randomized double-masked clinical trials that found topical natamycin to be superior to topical voriconazole and no additional benefit of adjuvant oral voriconazole. However, natamycin is fungistatic and has limited penetration into the corneal layers.6 Although topical natamycin is the best available treatment for moderate to severe fungal keratitis at this time, outcomes remain poor. In MUTT II, approximately 50% of study participants went on to full-thickness corneal perforation or required therapeutic penetrating keratoplasty (TPK). Therefore, we should continue to study other potential treatments or treatment combinations that may improve outcomes in fungal keratitis.

Voriconazole may still be an important adjunct in the treatment of fungal ulcers. In vitro studies suggest that voriconazole should have good efficacy against Aspergillus and Fusarium species. Intrastromal injection of
voriconazole may provide steady-state drug concentrations at the site of infection and avoid intervals of subtherapeutic drug dosing. Additionally, there may be presenting characteristics, such as deep stromal involvement, that could predict a benefit from intrastromal voriconazole (ISV) injection. However, studies of ISV have yielded mixed results. In this study, we evaluated the effectiveness of ISV injection in addition to topical natamycin treatment for the treatment of moderate to severe fungal keratitis.

Methods

Trial Design

The Mycotic Antimicrobial Localized Injection study was an institutionally funded randomized, outcome-masked, 2-arm clinical trial comparing clinical outcomes in study participants with moderate to severe smear-positive filamentous fungal corneal ulcers randomized to topical natamycin plus ISV injection versus topical natamycin alone. All study participants received medical therapy with topical natamycin 5%, eye drops (Aurolab, Madurai, India) every hour while awake, topical moxifloxacin 0.5% (Aurolab) for prophylaxis every 2 hours while awake, and homatropine 2% (Aurolab) 3 times daily.

Ethical approval was obtained from the University of California, San Francisco, Committee on Human Research and Aravind Eye Care System Institutional Review Board, Pondicherry, India. Written informed consent was obtained from all participants, and the trial conformed to the tenets of the Declaration of Helsinki. The data safety and monitoring committee recommended 1 interim analysis to review safety, data quality, and trial conduct. The study is registered at clinicaltrials.gov (identifier, NCT02731638).

Outcomes

The primary outcome of the trial was microbiological cure at 3 days on repeat culture analysis. Secondary outcomes included best spectacle-corrected visual acuity (BSCVA) at 3 weeks and 3 months; infiltrate or scar size, or both, at 3 weeks and 3 months; microbiological cure at 7 days; and adverse event, including corneal perforation or the need for TPK.

Study Participants

All study participants were enrolled at Aravind Eye Hospital in Pondicherry, India. Consecutive patients who sought treatment for smear-positive corneal ulcers with visual acuity of 20/70 (0.54 logarithm of the minimum angle of resolution [logMAR]) or worse were screened. Exclusion criteria included evidence of concomitant infection with herpes or bacteria, impending or frank perforation or limbal involvement, no light perception vision in the affected eye or visual acuity worse than 20/200 in the unaffected eye, age younger than 18 years or older than 70 years, and patients who were cognitively impaired or unable to complete follow-up. For the purposes of this study, a moderate ulcer was defined as a 2- to 6-mm ulcer in a central or peripheral location involving the anterior two thirds of the corneal stroma, and a severe ulcer was defined as one larger than 6 mm and involving the posterior stroma, with an endothelial plaque, or both.

Interventions

After eligibility was confirmed and written informed consent was obtained, patients were randomized in a 1:1 fashion to receive ISV injection plus topical natamycin 5% (Aurolab) versus topical natamycin 5% alone. For those randomized to intrastromal injections, the procedure was scheduled in the operating room within 24 hours of enrollment in the study. Using aseptic techniques, voriconazole 0.5 mg/ml solution (reconstituted with 2 ml of lactated Ringer’s solution; Vozole PF, Aurolab) was prepared just before administration and loaded into a 1-ml tuberculin syringe with a 30-gauge needle. With the bevel down, the needle was inserted obliquely starting in the adjacent uninvolved stroma to reach the infiltrate at the mid-stromal level (at the intended level for drug deposition). The drug was then injected and the amount of hydration of the cornea was used as a guide to assess the area saturated with medication. Four to 6 divided doses were administered around the infiltrate(s) to surround the entire circumference of the lesion(s). After the 3-day repeat corneal scraping, an additional 2 rounds of intrastromal injections were performed at 3 and 5 days after enrollment in the voriconazole injection arm only. All patients were hospitalized for the first 7 days so that all medications were observed directly and recorded by a health technician.

Study participants were examined by masked study physicians (S.N. and S.R.) at baseline, 3 days, 1 week, 1 month, and 3 months. A calibrated slit-lamp biomicroscope was used to assess the epithelial defect size; infiltrate or scar dimensions, or both; and depth according to a protocol adapted from the Herpetic Eye Disease Study. The presence of corneal perforation, hypopyon, or other ocular adverse events was also recorded. The study ophthalmologists were certified to ensure adherence to the study protocol. The patients were queried regarding serious and nonserious systemic adverse events.

Microbiological methods used for this study were adapted from a protocol used in the MUTT I that were published in detail previously. Baseline, 3-day, and 7-day scraping and culture samples were obtained from the corneal ulcers of all study participants. Corneal scraping was performed with a spatula using an aseptic technique and plated onto 2 separate microbiology slides for gram stain and potassium hydroxide wet mount. Three further scrapings were inoculated directly onto sheep’s blood agar, chocolate agar, potato dextrose, or Sabouraud’s agar for both bacterial and fungal culture analysis. Positive fungal smear results were defined as fungal elements seen under low-power magnification and reduced light. Positive fungal culture results were defined as light growth on any 2 media or moderate to heavy growth on 1 medium.

Best spectacle-corrected visual acuity was recorded at 4 m at enrollment, 3 weeks, and 3 months by a masked refractionist certified for the study using a protocol adapted from the Age-Related Eye Disease Study using Early Treatment Diabetic Retinopathy Study tumbling E charts (charts 2305 and 2305A; Precision Vision, Woodstock, IL). Low-vision testing was also performed at a distance of 0.5 m.

Masking

Study participants were not masked to their intervention but were asked not to share this information with any of the study personnel. Although the surgeon performing intrastromal injection was not masked because of the nature of the intervention, the study physician performing repeat scraping and outcome assessment remained masked to the treatment arm. The refractometric performing BSCVA and microbiologist analyzing culture results were also masked to treatment arm.

Statistical Analysis

The sample size was determined based on the primary end point of microbiological cure at 3 days. We anticipated that we would have 80% power to detect a difference in repeat culture status of 25% assuming a culture-positive rate of 50% in the control group and
25% in the intrastromal voriconazole group at 3 days with a 2-tailed \( p \) of 0.05% and no loss to follow-up (because participants were hospitalized after enrollment until the 3-day repeat culture samples were obtained). Participants were randomized using random block sizes in Microsoft Excel (Microsoft, Redmond, WA).

Baseline characteristics between the 2 arms were compared using the Fisher exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. The prespecified primary analysis used a logistic regression model to assess microbiological cure at 3 days between groups (dichotomous culture-positive or culture-negative outcome) controlling for baseline culture status. Cox proportional hazards regression models were used to estimate the hazard of perforation or need for TPK associated with intrastromal injection plus medical therapy versus medical therapy alone while correcting for baseline infiltrate or scar size, or both, as a fixed effect. An identical Cox proportional hazards regression model with an interaction term for organism and treatment arm was used to evaluate the effect of intrastromal voriconazole on the prespecified organism subgroups of Aspergillus species, Fusarium species, and all other organisms. A Wald test was performed to assess the significance of this interaction. Multiple linear regression was used to analyze BSCVA and infiltrate or scar size, or both, measured at 1 and 3 months with baseline measurements as covariates. The Fischer exact test was used to compare adverse events between arms.

For missing data for BSCVA resulting from TPK, we used the last observation carried forward or of 1.7 logMAR (approximate Snellen equivalents) before TPK was used. All analyses were conducted using Stata software version 13 (StataCorp, College Station, TX) and performed from October 5 through 12, 2018.

**Results**

A total of 151 patients with smear-positive ulcers were screened between October 7, 2016, and July 25, 2018, and 70 were randomized to topical natamycin 5% alone versus topical natamycin plus ISV injection (Fig S1, available at www.aaojournal.org). All study participants were enrolled at the Aravind Eye Hospital, Pondicherry, India. Follow-up for the primary outcome of 3-day repeat culture was available for 69 of 70 study participants (99%), and 3-month follow-up was available for 58 of 70 study participants (83%). For those lost to follow-up, home visits were performed to gather additional data. There was no evidence that loss to follow-up was associated with baseline visual acuity or treatment arm. Baseline participant demographics and clinical characteristics are outlined in **Table 1**. No major differences between groups were identified.

Organisms isolated from baseline cultures are described in **Table 2**. *Fusarium* species grew in 19 culture samples (27%), *Aspergillus* species grew in 17 culture samples (24%), other filamentous fungi grew in 19 culture samples (27%), and culture results were negative in 13 samples (19%). Baseline corneal culture results were negative in 5 (14%) of the topical natamycin only group and 8 (23%) of the ISV injection plus natamycin group.

At 3 days, 23 culture samples (68%) showed negative results in the natamycin only group and 21 (60%) showed negative results in the natamycin plus ISV injection group. Those randomized to ISV injection showed 1.44 times the odds of 3-day positive culture results \( (n = 69); 95\% \text{ confidence interval}[CI], 0.55–3.83; P = 0.46, \text{ bias-corrected logistic regression}) \) in posttest-only analysis and 1.82 times the odds of 3-day culture positivity if baseline culture status was included in the model \( (95\% \text{ CI}, 0.65–5.23; P = 0.26, \text{ bias-corrected logistic regression}) \). Those receiving
ISV injections had 1.98 times the odds of positive 7-day culture results after controlling for baseline culture status (95% CI, 0.69–5.91; \( P = 0.20 \), bias-corrected logistic regression). If the patient’s baseline culture results were positive for Fusarium species (n = 19), those randomized to ISV injection had 3.33-fold increased odds of 3-day culture positivity (95% CI, 0.25–45.11; \( P = 0.37 \)). If the patient’s baseline culture results were positive for Aspergillus species (n = 16), those randomized to ISV had 0.75-fold increased odds of 3-day culture positivity (95% CI, 0.04–14.58; \( P = 0.85 \)). If the patient’s baseline culture results were positive for any other filamentous fungus and they were randomized to the ISV injection group, they had 3.6-fold increased odds of 3-day repeat culture positivity (95% CI, 0.49–26.39; \( P = 0.21 \)).

Mean 3-week visual acuity was 1.23 logMAR (standard deviation, 0.65 logMAR) in the natamycin only arm and 1.40 logMAR (standard deviation [SD], 0.59 logMAR) in the ISV injection arm. Mean 3-month visual acuity was 1.28 logMAR (SD, 0.66 logMAR) in the natamycin arm and 1.30 logMAR (SD, 0.60) in the ISV injection plus natamycin arm. Those randomized to ISV injection showed 1.6 logMAR (approximately 1.5 Snellen lines) worse visual acuity at 3 weeks after controlling for baseline visual acuity (95% CI, −1.2 to 4.4 logMAR; \( P = 0.25 \)) and 0.5 logMAR (approximately 0.5 Snellen line) decreased visual acuity after controlling for baseline visual acuity (95% CI, −2.6 to 3.6 logMAR; \( P = 0.75 \)).

At 3 weeks, the infiltrate or scar size or both was 0.69 mm larger among those who were randomized to ISV injection after controlling for baseline measurements (95% CI, 0.04–1.33 mm; \( P = 0.04 \)). Multiple linear regression models also found 0.55 mm worse infiltrate or scar size or both at the 3-month infiltrate among those receiving ISV injection. After controlling for baseline, this was no longer statistically significant (95% CI, −0.13 to 1.25 mm; \( P = 0.11 \)).

Adverse events are outlined in Table 3. Overall, 11 study participants (16%) experienced full-thickness corneal perforation, 8 (23%) in the ISV injection arm and 3 (9%) in the natamycin-only arm. In a Cox proportional hazard model, those randomized to ISV injection showed a 2.85-fold increased hazard of perforation after controlling for baseline infiltrate depth (95% CI, 0.76–10.75; \( P = 0.12 \)). Twenty-seven study participants (39%) eventually required TPK, including 14 (40%) in the ISV injection arm and 13 (37%) in the natamycin-only arm. In a Cox proportional hazards model, those randomized to ISV injection exhibited a relative hazard of 0.95 for eventually requiring TPK after controlling for baseline infiltrate or scar depth or both (95% CI, 0.44–2.04; \( P = 0.90 \)). Intrastromal voriconazole injection conferred a 1.12-times increased hazard of experiencing any adverse event, but this was not statistically significant (95% CI, 0.44–2.88; \( P = 0.81 \)).

**Discussion**

We found no benefit to adjuvant ISV in this randomized clinical trial comparing ISV injection plus topical natamycin 5% with natamycin 5% alone for the primary treatment of moderate to severe filamentous fungal ulcers. Specifically, we found no improvement in microbiological cure rate at 3 or 7 days. Studies have suggested that in addition to providing an initial diagnosis, repeat culture analysis can be used to assess response to treatment and is correlated highly with clinical outcomes such as visual acuity. Surrogate outcomes such as these have become increasingly common in infectious disease trials.\(10^{–19}\) Supporting our microbiological outcomes, we also found no improvement in visual acuity and no reduction in the rate of perforation or need for TPK among those randomized to ISV injection. Scar size may be increased among those receiving ISV injection. In prespecified subgroups looking at Fusarium species, Aspergillus species, and other filamentous fungi, we found no evidence of a benefit to adjuvant ISV injection.

Although in vitro studies have suggested that voriconazole showed good efficacy against filamentous fungi, clinical studies have been less encouraging. MUTT I demonstrated that topical voriconazole was inferior to topical natamycin, whereas MUTT II showed no benefit to adjuvant oral voriconazole in addition to topical natamycin. Theoretically, adjuvant ISV could be advantageous given natamycin’s limited penetration into deep corneal stroma.

<table>
<thead>
<tr>
<th>Table 2. Baseline Microbiologic Culture Results</th>
<th>Standard Therapy (n = 34)*</th>
<th>Intrastromal Voriconazole (n = 34)*</th>
<th>Total (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium species</td>
<td>11 (31)</td>
<td>8 (23)</td>
<td>19</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>10 (29)</td>
<td>7 (20)</td>
<td>17</td>
</tr>
<tr>
<td>A. flavus</td>
<td>9 (26)</td>
<td>7 (20)</td>
<td>16</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Curvularia species</td>
<td>0 (0)</td>
<td>4 (11)</td>
<td>4</td>
</tr>
<tr>
<td>Exserohilum species</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>2</td>
</tr>
<tr>
<td>Unidentified hyaline</td>
<td>2 (6)</td>
<td>3 (9)</td>
<td>5</td>
</tr>
<tr>
<td>Unidentified dematiaceous</td>
<td>5 (14)</td>
<td>3 (9)</td>
<td>8</td>
</tr>
<tr>
<td>Fungal culture negative</td>
<td>5 (14)</td>
<td>8 (23)</td>
<td>13</td>
</tr>
</tbody>
</table>

Data are no. (%). *Missing data for 1 patient in each arm.

<table>
<thead>
<tr>
<th>Table 3. Adverse Events by Treatment Group</th>
<th>Standard Therapy (n = 35)</th>
<th>Intrastromal Voriconazole (n = 35)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse Event</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endophthalmitis</td>
<td>2 (6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Glaucoma</td>
<td>2 (6)</td>
<td>4 (11)</td>
<td></td>
</tr>
<tr>
<td>Hypopyon</td>
<td>0 (0)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Medication reaction</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Nonhealing ulcer</td>
<td>0 (0)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Perforation</td>
<td>3 (9)</td>
<td>8 (23)</td>
<td></td>
</tr>
<tr>
<td>Progressive corneal thinning</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Therapeutic penetrating keratoplasty</td>
<td>13 (37)</td>
<td>14 (40)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>33</td>
<td>0.81*</td>
</tr>
</tbody>
</table>

Data are no. (%) unless otherwise indicated.

*Fisher exact test comparing number of people with any adverse event in each arm.
Several prior case series have suggested that it could be beneficial.\textsuperscript{3–10} One prior randomized controlled trial comparing intrastromal injection with topical voriconazole found significantly improved 3-month visual acuity in the topical voriconazole group.\textsuperscript{11} However, these results were difficult to interpret, because in the intrastromal voriconazole arm, there were more central ulcers than in the topical voriconazole arm, and final scar size between the 2 groups was comparable, implying that intrastromal injection did not lead to worse scarring.

Limitations to this study include the fact that all patients enrolled in this study were from India, and most infections were related to agricultural exposure and not to contact lens wear, such as those seen in developed countries. Therefore, it is possible that organisms in this study exhibit different response patterns to medications. Only a small number of each type of fungus was represented, which may have made it difficult to detect a benefit of ISV injection for any particular organism. This study was powered to detect a difference in microbiological cure, a surrogate endpoint. Studies have suggested that in addition to providing an initial diagnosis, repeated culture can be used to assess response to treatment and is correlated highly with clinical outcomes such as visual acuity, and outcomes such as these have become increasingly common in infectious disease trials.\textsuperscript{16–19} Additionally, clinical outcomes such as visual acuity, scar size, and rate of perforation and TPK in this study were supportive of the primary outcome.

In conclusion, there seems to be no benefit to adding ISV injections to topical natamycin in the primary treatment of moderate to severe filamentous fungal ulcers. Studies consistently suggest that voriconazole has a limited role in the treatment of filamentous fungal ulcers.

Acknowledgment
A biostatistician graduate student at the Proctor foundation, Kathryn J. Ray, MS, performed the randomization.

References

2. Deorukhkar S, Katiyar R, Saini S. Epidemiological features and laboratory results of bacterial and fungal keratitis: a five-year study at a rural tertiary-care hospital in western Mahara-
13. Prajna NV, Krishnan T, Mascarenhas J, et al. The mycotic ulcer treatment trial: a randomized trial comparing natamy-
14. Age-Related Eye Disease Study Research Group. The Age-
16. Bladhage Y, Das S, Kasav MK, et al. Comparison of culture-
negative and culture-positive microbial keratitis: cause of culture negativity, clinical features and final outcome. *Br J Oph-

Footnotes and Financial Disclosures

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**Pictures & Perspectives**

**Tattoo Inflammation and Sarcoid Uveitis**

A 21-year-old African-American man in uniformed services presented for 6-weeks of bilateral eye inflammation nonresponsive to topical corticosteroids. Two weeks before the eye symptoms, his tattoos became elevated (Fig A, red arrows) and changed in color. He had active vitreous inflammation, macular edema (Fig B, short arrow), and optic disc edema (long arrows). An infectious and inflammatory work-up was negative. Computed tomography of the patient’s chest demonstrated calcified sub-carinal lymph nodes (Fig C, green arrow) confirming clinically suspected sarcoidosis. Skin biopsy showed non-caseating granulomas (Fig D, blue arrows) and multinucleated giant cells with pigment (D, yellow arrows). He is currently being managed on oral immunosuppressive agents. (Magnified version of Fig A-D is available online at www.aaojournal.org).

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