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Therapeutic Penetrating Keratoplasty Button Cultures in The Mycotic Ulcer Treatment Trial II: A Randomized Trial Comparing Oral Voriconazole Versus Placebo

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Abstract

OBJECTIVE—To compare oral voriconazole vs placebo in addition to topical antifungals in the treatment of filamentous fungal keratitis.

DESIGN—Non-prespecified, secondary case-control analysis from a multicenter, double-masked, randomized placebo-controlled clinical trial.

Study Participants—Patients with smear-positive filamentous fungal ulcers and visual acuity of 20/400 or worse who eventuated to therapeutic penetrating keratoplasty (TPK).

Intervention—Study participants were randomized to oral voriconazole vs oral placebo; all received topical antifungal drops.

Main Outcome Measures—TPK button culture positivity.

RESULTS—A total of 95 of 194 (49.5%) study participants enrolled at Madurai, Coimbatore, or Pondicherry, India eventuated to TPK in an average of 20.9 days (standard deviation 15.2 days, range 2–71 days). TPK button cultures were available for 67 of 95 (71%) of the TPKs performed and were positive for filamentous fungus in 45 of 67 (67%) cases. For each 1-day increase in the time to TPK there was 0.94-fold decreased odds of fungal culture positivity (95% confidence interval [CI] 0.90–0.98, $P = .005$). Those randomized to oral voriconazole had 1.26-fold increased odds of TPK button culture positivity after controlling for time to TPK and baseline organism, but this was not statistically significant (95% CI 0.32–4.87; $P = .74$). Those who underwent TPK for lack of response to medical therapy were 10.64-fold more likely to be culture positive than if the indication for surgery was perforation and this was statistically significant (95% CI 2.16–51.70; $P = .003$).

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Supplemental Material available at AJO.com.

CONCLUSIONS—There appears to be no benefit to adding oral voriconazole to topical antifungal agents in the treatment of severe filamentous fungal ulcers. Infection rather than inflammation appears to be the reason for the worsening clinical picture in many of these patients.

Corneal perforation and the need for therapeutic penetrating keratoplasty (TPK) occur frequently in fungal keratitis and the optimal treatment to prevent such complications is not yet known.[1] The Mycotic Ulcer Treatment Trial II (MUTT II) was unable to find a significant benefit to adding oral voriconazole to topical natamycin and topical voriconazole in reducing the risk of perforation or the need for TPK in severe filamentous fungal keratitis.[2,3] However, complications such as perforation may been a result of the robust inflammatory response to infection as well as to uncontrolled infection.[4] Therefore, oral voriconazole may have had an effect on the infection that was not detected clinically.

Culture positivity despite treatment has previously been found to be an important predictor of clinical outcomes in both bacterial and fungal keratitis.[5–7] Here, we compare the TPK button culture results by treatment arm to determine if we can detect a benefit of oral voriconazole microbiologically that we were unable to determine clinically.

METHODS

The methods for mutt ii have been outlined in detail in a previous publication.[2] In brief, MUTT II was a double-masked, placebo-controlled randomized clinical trial that enrolled 240 patients with smear-positive filamentous fungal corneal ulcers and visual acuity of 20/400 or worse. Institutional Review Board approval was obtained at the University of California, San Francisco, the Aravind Eye Care System, and the Dartmouth-Hitchcock Medical Center Committee for the Protection of Human Subjects. The trial (Trial Registration: NCT00996736) onformed to the Declaration of Helsinki and written informed consent was obtained from all participants.

Enrollment sites included multiple sites of the Aravind Eye Care System (Madurai, Pondicherry, Coimbatore, and Tirunelveli), the Lumbini and Bharatpur Eye Hospitals in Nepal, and the University of California, San Francisco. All participants were randomized to receive oral voriconazole or placebo in addition to topical antifungals. At the beginning of the trial, patients randomized to receive oral voriconazole were given a loading dose of 400 mg twice daily for 24 hours, and then a maintenance dose of 200 mg twice daily for 20 days. As of September 2010, after 14 study participants (6% of a projected 240) had been enrolled, weight-based dosing was introduced to the protocol in an attempt to reduce side effects. If patients weighed ≥50 kg, they were given the original oral voriconazole dose. If they were 40–50 kg, they received a 300 mg loading dose and 150 mg twice-daily (BID) maintenance dose thereafter and if <40 kg they received a 200 mg loading dose followed by 100 mg BID maintenance dose. The primary outcome of the trial was rate of perforation or the need for TPK.

In this non-prespecified secondary analysis, corneal button cultures from TPKs performed in MUTT II study participants at the Aravind Eye Hospital at Madurai, Pondicherry, and Coimbatore, India, were obtained. A logistic regression model assessed the relationship between adjuvant oral voriconazole and TPK button culture status (a binary outcome of

positive or negative) with covariates for time to TPK and baseline culture result. A regression model also analyzed the relationship between indication for TPK, classified as either perforation or lack of clinical response to medical therapy, and button culture status.

RESULTS

A total of 95 of the 194 mutt ii study participants (49.5%) enrolled at Madurai, Coimbatore, or Pondicherry, India, underwent TPK. Baseline cultures were positive for filamentous fungus in 80 of those 95 who eventuated to TPK (84.2%). These cultures isolated *Fusarium* spp *in* 36.8% (n = 35), *Aspergillus* spp in 28.4% (n = 27), and all other fungi in 18.0% (n = 18). Baseline cultures were negative in 15.8% (n = 15). The average time to TPK was 20.9 days (standard deviation 15.2 days, range 2–71 days). Table 1 outlines the baseline characteristics of study participants with TPK cultures by treatment arm.

Of the 95 TPKs performed, TPK button cultures were available for 67 (71%). TPK button cultures were positive for filamentous fungus in 45 of the 67 cases (67%) and grew *Fusarium* spp in 36% (n = 24), *Aspergillus* spp in 16% (n = 11), other fungi in 15% (n = 10), *Pseudomonas aeruginosa* in 3% (n = 2), and no growth in 22% (n = 20). Of the 15 fungal ulcers that were culture negative at baseline, the TPK button cultures were unavailable in 6 cases (40%). Of those that were culture negative at baseline and had TPK button cultures, 7 (77.8%) continued to show no growth, 1 (11.1%) grew *Fusarium*, and 1 (11.1%) grew other filamentous fungi.

For each 1-day increase in the time to TPK there was 0.94-fold decreased odds of fungal culture positivity (95% confidence interval [CI] 0.90–0.98, $P = .005$). TPK button cultures were positive in 41.7% of TPK buttons after 1 month of antifungal therapy, 37.5% after 6 weeks, and 25.0% after 8 weeks (range of culture positive 2–56 days post enrollment). In univariate analysis, baseline patient characteristics such as age ($P = .15$), sex ($P = .18$), isolated organism ($P = .17$), epithelial defect size ($P = .06$), infiltrate and/or scar size ($P = .19$), and ulcer depth ($P = .07$) did not predict TPK button culture positivity.

Table 2 outlines the odds of culture positivity by treatment arm and by indication for surgery. Excluding the 2 cases that were culture positive for *Pseudomonas*, those randomized to oral voriconazole had 1.26-fold increased odds of TPK button culture positivity after controlling for time to TPK and baseline organism, but this was not statistically significant (95% CI 0.32–4.87; $P = .74$). There was also no evidence that oral voriconazole reduced the odds of culture positivity in *Fusarium* spp ulcers (coefficient 7.50, 95% CI 0.33–172.83; $P = .21$).

TPKs that were performed for perforation had positive TPK cultures in 39% (9/23) while those performed for lack of response to medical therapy were positive in 82% (36/44). After controlling for baseline culture results, time to TPK, and treatment arm, those who underwent TPK for lack of response to medical therapy (as determined by the masked ophthalmologist) were 10.64-fold more likely to be culture positive than if the indication for surgery was perforation, and this was statistically significant (95% CI 2.16–51.70; $P = .003$).

DISCUSSION

We were unable to demonstrate a difference in tpk culture status between those randomized to oral voriconazole those randomized to placebo. This microbiological result is consistent with the overall MUTT II clinical finding that adjuvant oral voriconazole was not a benefit in the treatment of severe fungal keratitis.[2] We did find that study participants who underwent TPK for lack of clinical response to medical therapy were 10-fold more likely to be culture positive than those whose indication was perforation. This outcome suggests that the worsening clinical picture in many of these patients is attributable to infection rather than the inflammatory response.

Our series also illustrates the severe nature of fungal keratitis and the importance of continued research into its optimal treatment. Fully two thirds of our TPK buttons were culture positive and although there were decreasing odds of being culture positive for each day delay in proceeding to TPK, some study participants had positive TPK cultures up to 56 days after enrollment in the study and initiation of antifungal therapy. A prior study of TPK cultures and histopathology in fungal keratitis found that 42% of TPK buttons were culture positive after 2 months.[8] Microbiological cure has been associated with favorable clinical outcomes in infectious diseases such as pneumonia.[9] Repeat culture positivity despite appropriate antifungal therapy has been correlated with poor clinical outcomes in fungal keratitis, such as worse visual acuity and larger scar size.[5] Therefore, the high rate of culture positivity in our series, combined with the fact that these patients were treated with maximum medical therapy (both topical natamycin and voriconazole, with roughly half randomized to oral voriconazole), underscores the importance of evaluating other potential therapies such as corneal cross-linking or early TPK.[10]

Limitations to this study include the fact that we enrolled severe ulcers; therefore it may have been difficult to detect a difference between groups owing to the advanced nature of the infection. Because this was a non-prespecified analysis, TPK cultures were only available for 71% of TPKs at Aravind. Although this could be a potential source of bias, the available cultures were balanced between treatment groups, and baseline characteristics were similar to the overall MUTT II study. We used weight-based dosing of voriconazole. Efficacy of oral voriconazole may be related to serum concentration. Monitoring a voriconazole trough would have verified that our study subjects had the levels necessary to ensure drug efficacy. Only small numbers of each organism were represented in this study, which may make it difficult to draw conclusions about the best treatment for each organism. Most of the infections in this study were related to agricultural exposure and not contact lens wear, such as those seen in developed countries.

In conclusion, microbiological evidence from TPK cultures supports the finding of MUTT II that there was not a benefit to adjuvant oral voriconazole in treating severe filamentous fungal keratitis. Worsening infection rather than inflammation appears to be the reason for the worsening clinical picture in many of these patients. Given this finding, along with the increased risk of systemic side effects and cost associated with oral voriconazole, we cannot recommend the adjuvant use of oral voriconazole for the treatment of severe filamentous fungal keratitis at this time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Baseline Characteristics by Treatment Arm in Subjects With Therapeutic Penetrating Keratoplasty Cultures

Baseline Characteristic	Placebo N = 38	Oral Voriconazole N = 29
Male sex, n[a]	20 (52.6%)	15 (51.7%)
Age (years), mean (SD)	52.1 (13.1)	54.1 (12.8)
Affected eye, right, n	23 (60.5%)	18 (62.1%)
LogMAR visual acuity, mean (SD)	1.6 (0.37)	1.7 (0.30)
Infiltrate/scar[b] (mm), mean (SD)	6.17 (1.28)	6.13 (1.28)
% of depth, n		
>0 to 33%	7 (18.4%)	3 (10.3%)
>33%–67%	11 (29.0%)	11 (37.9%)
>67%–100%	19 (50.0%)	15 (51.7%)
Epithelial defect[b] (mm), mean (SD)	5.62 (1.52)	4.99 (1.96)
Baseline culture		
Fusarium	16 (42.1%)	11 (38.0%)
Aspergillus	9 (23.7%)	6 (20.7%)
Other fungus	8 (21.1%)	8 (27.6%)
Culture negative	5 (13.2%)	4 (13.8%)
Time to TPK, days (SD)	22 (16)	21 (15)

LogMAR = logarithm of the minimal angle of resolution; TPK = therapeutic penetrating keratoplasty.

[a] Sex information not available for 1 study participant.

[b] Geometric mean of the longest diameter and longest perpendicular to that diameter in millimeters.

TABLE 2

Therapeutic Penetrating Keratoplasty Culture Positivity by Treatment Arm and Indication for Surgery

Characteristic	Odds	95% CI	*P* Value
Oral voriconazole[a]	1.26	0.32–4.87	.74
Fusarium spp	7.50	0.85–172.83	.21
Aspergillus spp	0.27	0.01–6.48	.42
Other fungi	0.40	0.03–6.35	.52
Indication for Surgery[b]	10.85	2.11–55.68	<.001

[a]Logistic regression model looking at all fungi by treatment arm with covariates for time to therapeutic penetrating keratoplasty (TPK) and baseline culture result.

[b]Logistic regression model looking at all fungi by indication for surgery, either perforation or lack of response to medical therapy, with covariates for time to TPK and baseline culture result.