Brief Report

Changing Azole Resistance
A Secondary Analysis of the MUTT I Randomized Clinical Trial

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IMPORTANCE The development of multiple triazole resistance in pathogenic filamentous fungi has become an increasing clinical concern and has been shown to increase the risk for treatment failure.

OBJECTIVE To determine whether antifungal resistance increased during the Mycotic Ulcer Treatment Trial I (MUTT I), as measured by minimum inhibitory concentrations (MICs) in baseline cultures.

DESIGN, SETTING, AND PARTICIPANTS This secondary analysis of a double-masked, multicenter, randomized clinical trial included patients with culture- or smear-positive filamentous fungal corneal ulcer and a baseline visual acuity of 20/40 to 20/400. Culture-positive samples with susceptibility testing were included in this analysis. The patients were treated at multiple locations of the Aravind Eye Care Hospital system in South India. Data were collected from April 3, 2010, to December 31, 2011, and analyzed from July 15 to September 1, 2015.

INTERVENTIONS Corneal smears and cultures were obtained from all study participants at baseline. Susceptibility testing was performed for each culture-positive specimen.

MAIN OUTCOMES AND MEASURES Minimum inhibitory concentration of voriconazole and natamycin in baseline cultures.

RESULTS Of 323 participants with smear-positive specimens (183 men [56.7%]; 140 women [43.3%]; median [interquartile range] age, 47 [38-56] years), fungal-positive cultures were obtained for 256 (79.3%). The MIC data were available for 221 of 323 participants (68.4%), because 35 samples had no growth during susceptibility testing. A 2.14-fold increase per year (95% CI, 1.13-4.56; $P = .02$) in voriconazole MICs after controlling for the infectious organism was found. This association was not found when looking at natamycin MICs of baseline cultures after controlling for the infectious organism (1.26; 95% CI, 0.13-12.55; $P = .85$).

CONCLUSIONS AND RELEVANCE Susceptibility to voriconazole appeared to decrease during the relatively short enrollment period of the clinical trial. This decrease may be more related to increased resistance of environmental fungi rather than previous treatment with azoles, because presenting with azole treatment was not a risk factor for resistance.

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The development of multiple triazole resistance among pathogenic filamentous fungi has become an increasing clinical concern and has been shown to increase the risk for treatment failure.[1] The mechanism for azole resistance in filamentous fungi may be a mutation in the *CYP51* gene responsible for producing the lanosterol-14α-demethylase enzyme, which is the drug target.[2] Development of resistance has been documented in patients with chronic fungal infections such as pulmonary aspergillosis after prolonged use of triazole antifungals.[3] However, specimens have also been cultured from azole-naïve patients, which suggests that the azole-resistant organisms were acquired in the environment.[4,5] India has reported the emergence and spread of azole-resistant fungi in the environment owing to widespread agricultural use.[3,6] Recovery of azole-resistant filamentous fungi from soil samples in India has increased dramatically from 0% to 5% in 2002 to 2004 to 17% to 20% just 5 years later.[3,6]

The Mycotic Ulcer Treatment Trial I (MUTT I), a double-masked, randomized clinical trial funded by the National Institutes of Health, found natamycin to be superior to voriconazole in the treatment of filamentous fungal ulcers, and in particular those infected with *Fusarium* species.[7] Secondary analyses collected from MUTT I data showed that a 2-fold increase in minimum inhibitory concentration (MIC) was associated with increased odds of perforation and increased 3-month scar size, but not decreased visual acuity.[1] In this nonprespecified subgroup analysis, we investigate how azole resistance patterns changed during MUTT I.

Key Points

Question Did antifungal drug resistance increase during the Mycotic Ulcer Treatment Trial I?

Findings The decrease in susceptibility to voriconazole during the clinical trial was statistically significant. Resistant strains have been cultured in azole-naïve patients, suggesting increasing resistance of environmental fungi.

Meaning A trend of increasing azole resistance could affect the treatment of human mycoses.

Methods

Detailed MUTT I methods have been published previously.[7,8] Briefly, patients with smear-positive filamentous fungal corneal ulcers and visual acuity ranging from 20/40 to 20/400 who were enrolled at Aravind Eye Care System (Madurai, Pondicherry, and Coimbatore) were randomized to receive topical natamycin, 5%, or topical voriconazole, 1%. The primary MUTT I outcome was the 3-month best spectacle-corrected visual acuity; prespecified secondary outcomes included 3-month infiltrate and/or scar size, time to reepithelialization, and corneal perforation or the need for therapeutic penetrating keratoplasty. The trial was approved by the institutional review boards of the Aravind Eye Care System, Dartmouth Medical School, and the University of California, San Francisco. The study adhered to the guidelines in the Declaration of Helsinki.[9] All patients provided written informed consent.

Data were collected from April 3, 2010, to December 31, 2011. Microbiologic methods used in MUTT I have been described in detail previously.[10] Baseline scrapings and cultures were obtained from the corneal ulcers of all study participants. Using the standards set by the Clinical and Laboratory Standards Institute, speciation and analysis of natamycin and voriconazole MICs were performed for all positive fungal samples collected. The MIC was defined as the lowest concentration of antifungal required to reduce solution turbidity by 100%.

Data were analyzed from July 15 to September 1, 2015. Log_2-transformation of the MIC as a continuous variable was used for all statistical models. In this non-prespecified subgroup analysis, we performed multiple linear regression to analyze the association between the date of enrollment in the study as our primary predictor of interest and the baseline MIC, adjusted for the infectious organism. This process resulted in a change in log_2-tranformed voriconazole MIC per day. To obtain the fold change in MIC per year, we took the antilog of the coefficient multiplied by 365. The infectious organism (*Aspergillus, Fusarium*, and other species) was treated as a categorical variable. We also performed sensitivity analyses looking at the effects of pretreatment and of autocorrelation of temporally adjacent observations on the final statistical model. Unless otherwise specified, data were expressed as mean (SD).

Results

From April 3, 2010, to December 31, 2011, positive fungal cultures were obtained from 256 of 323 study participants (79.3%) with smear-positive specimens. The MIC data were available for 221 of 323 participants (68.4%), because 35 samples had no growth during susceptibility testing. The mean voriconazole MIC for all organisms during the entire study was 3.19 (3.62) μg/mL. Mean voriconazole MICs were 4.69 (3.79) μg/mL for *Fusarium* species, 0.99 (1.34) μg/mL for *Aspergillus* species, and 1.47 (2.83) μg/mL for all other organisms. Mean voriconazole MICs for all organisms increased from 1.86 (1.87) μg/mL in 2010 to 3.79 (4.04) μg/mL in 2011.

Results of multivariate linear regression giving fold change in MIC per year are outlined in the **Table**. A 2.14-fold increase per year (95% CI, 1.13- 4.56; $P = .02$) was found in the voriconazole MIC after controlling for the infectious organism. Controlling for pretreatment status did not change the results of the analysis appreciably. After accounting for autocorrelation of voriconazole MICs, the increase in voriconazole MIC per year was 1.97 (95% CI, 1.41-2.76; $P < .001$); after correcting for the organism, the increase in MIC was 1.29 (95% CI, 1.14-1.61; $P = .046$). This association was not found for natamycin MICs for baseline cultures after controlling for the infectious organism (1.26; 95% CI, 0.13-12.55; $P = .85$).

Table. Multiple Linear Regression Predicting Voriconazole Susceptibility

Organism	No. (%) of Participants	Fold Change in MIC		*P* Value
		Mean (SD)	Fold Increase per Year (95% CI)[a]	
All	221 (100)	3.19 (3.62)	2.14 (1.13-4.56)	.02
Subgroup species				
Aspergillus	52 (23.5)	0.99 (1.34)	0.98 (0.60-1.66)	.95
Fusarium	126 (57.0)	4.69 (3.79)	3.48 (1.03-9.75)	.045
Other	43 (19.5)	1.47 (2.83)	2.75 (0.78-7.57)	.13

Abbreviation: MIC, minimum inhibitory concentration.

[a] Multiple linear regression used the date of enrollment in days and the organism as predictors, with the slope converted to years.

Discussion

We found an increase in azole resistance of filamentous fungi recovered from baseline corneal cultures during MUTT I after controlling for the infectious organism. Azole resistance has important clinical implications because azole antifungals are some of the most commonly used fungicides to control human fungal pathogens. Systemic aspergillosis with azole-resistant strains has an extremely poor prognosis.[3] Reduced success in the treatment of corneal ulcers has also been associated with increasing MICs to voriconazole.[1] Treatment with azoles before presentation and enrollment in MUTT I was not shown to increase voriconazole MICs.[11] This finding suggests that the increased resistance patterns observed in this study are not owing to treatment of disease with antifungals.

Another potential mechanism that would explain our observation is the rapidly increasing resistance of fungi recovered from soil samples in India owing to the widespread use of azoles in agriculture.[4,6] Among MUTT I study participants, 48% were agricultural workers and 64% experienced ocular trauma with vegetative material as the main risk factor for the fungal keratitis. By contrast, increasing resistance to polyenes, such as natamycin, was not observed in our study or generally in clinical infections. This finding may reflect inherent differences in the ability of fungi to acquire resistant mutations to polyenes or may be owing to the fact that polyenes are rarely used in agriculture. Concerns about the contribution of agricultural practices to the development of azole resistance in clinical infections are not isolated to India, because similar observations have been seen in other parts of the world.[5]

In subgroup analyses looking at individual organisms, we were unable to detect the development of *Aspergillus* resistance. We found evidence of acquisition of *Fusarium* resistance. Although *Fusarium* infection outside the eye is rare in humans, it is an emerging pathogen among immunocompromised patients, and the development of resistance to azoles in vitro has been described.[12]

Our study has several limitations. We performed a nonprespecified, hypothesis-generating analysis, and therefore this question requires further study. The causative organisms found in our study participants likely exhibit different in vitro and in vivo activity. The small numbers of each organism in the study make comparisons difficult. In addition, the study duration was only 20 months, which has likely reduced our ability to detect the development of resistance.

Conclusions

Susceptibility to voriconazole apparently decreased during the relatively short enrollment period of a clinical trial. These microbiologic findings highlight the primary clinical trial result that natamycin is superior to voriconazole in the treatment of filamentous fungal keratitis. A trend of increasing azole resistance could affect treatment of human mycoses.

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