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Does *in vitro* susceptibility predict clinical outcome in bacterial keratitis?

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Abstract

Purpose—To determine whether clinical outcomes in bacterial keratitis are associated with antibiotic susceptibility.

Design—Retrospective ancillary study using data and samples from a completed randomized clinical trial.

Methods—42 patients were enrolled with culture-confirmed bacterial keratitis at Aravind Eye Hospital in South India. All patients received topical moxifloxacin and were randomized to receive either topical prednisolone phosphate or placebo. Outcomes included time to epithelialization, best spectacle-corrected visual acuity (BSCVA) and infiltrate-scar/size at 3 months. Bacterial isolates were cultured and minimum inhibitory concentration (MIC) to moxifloxacin was measured using Etests. Multiple linear regression was used to assess the effect of MIC on outcome, adjusting for enrollment characteristics.

Results—MIC was associated with 3-month infiltrate/scar size: each two-fold increase in MIC was associated with a 0.33 mm average diameter increase in scar size (p=0.01). MIC was not associated with 3-month BSCVA (p=0.71) or time to epithelialization (p=0.35).

Conclusions—MIC was associated with infiltrate/scar size in bacterial keratitis. An ongoing larger, multi-center trial should provide further information on whether this association is maintained across subgroups of organisms.

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Introduction

Infectious keratitis is the major cause of corneal blindness in the U.S., with over 30,000 bacterial ulcers per year.[1] This number is far greater in developing countries.[2, 3] Even with the current standard of care, it is estimated that 25% of patients are left legally blind after treatment.[4] One important clinical question is whether tailoring antibiotic therapy based on microbial sensitivity data can improve outcomes. Bacterial susceptibility to antibiotics is routinely measured, but its association to visual outcome for the most common agents currently in use has not been well studied. For systemic infections, it has been suggested that susceptibility and outcome are associated, following a "90-60 rule."[5, 6] Sensitive bacteria respond to therapy approximately 90% of the time, whereas resistant bacteria respond to therapy 60% of the time. This relationship may not necessarily hold in ocular infections, particularly since there are no established standards for assessing susceptibility specific to antibiotic concentrations established in the cornea with topical treatment. Ophthalmic preparations are applied directly at the target site at concentrations more than 1000-fold higher than that necessary to inhibit the organism *in vitro*. On the other hand, systemic antibiotics take hours to achieve a peak concentration, and their concentrations at the target site are typically diluted.

The role of antibiotic susceptibility testing in the care of bacterial keratitis is unclear, especially given the current widespread use of broad-spectrum fluoroquinolones such as gatifloxacin and moxifloxacin.[7, 8] Minimal inhibitory concentration (MIC) is a measure of antibiotic susceptibility, and the Etest has been validated as a convenient and accurate alternative to the broth dilution method for measuring MIC.[9, 10] Our objective was to determine whether MIC is associated with clinical response in bacterial keratitis and could potentially serve as a useful guide in determining therapy.

Methods

This study utilized samples and data collected in a pilot study conducted in preparation for the Steroids for Corneal Ulcers Trial (SCUT). The SCUT pilot was a randomized, double-masked clinical trial conducted at the Aravind Eye Hospital in Madurai, India with the primary objective to assess the effect of topical corticosteroid treatment on bacterial corneal ulcers. Aravind Eye Hospital is both a primary and a tertiary care eye hospital in South India with a well-established cornea subspecialty clinic. The target enrollment for the study was 42 patients, and the study has now been completed. All study patients with culture-confirmed bacterial keratitis were randomized to a 3-week course of topical prednisolone phosphate 1% or placebo. Patients received topical moxifloxacin (Vigamox®) treatment 1 drop every hour for 48 hours while awake, then every 2 hours until re-epithelialization, and then 4 times a day until 3 weeks after enrollment. Moxifloxacin was donated by Alcon (Fort Worth, TX). Patients were admitted until re-epithelialization ensuring timely delivery of antibiotics and study medication. Best spectacle-corrected visual acuity (BSCVA) and infiltrate-scar size were performed at enrollment, 3 weeks and 3 months.

Bacterial isolates from the patients enrolled in the trial were tested for susceptibility to moxifloxacin using the Etest method (AB BIODISK, Solna, Sweden). All isolates were stored in a -70°C freezer and subcultured using organism-specific culture media. Quality control was performed for the Etest according to the Clinical and Laboratory Standards Institute (CLSI) protocol.[11] Etests were performed to obtain MIC readings and were repeated on all of the isolates on a different day to obtain an average MIC for the analysis. The test observer was masked to treatment arm, clinical outcomes, and any prior reading of MIC.

BSCVA was expressed in logMAR units. Low vision was assigned the same logMAR readings used in the Herpetic Eye Disease Study (HEDS): counting fingers (logMAR=1.7), hand motion (1.8), light perception (1.9), and no light perception (2.0).[12] Infiltrate-scar sizes were estimated by slit lamp exam using the same technique as in HEDS, multiplying the longest linear diameter and the longest perpendicular.[12] The geometric mean of the two measurements was recorded for analysis. MICs are known to have a skewed distribution, so all analyses used log-transformed MIC. Multiple linear regression was performed predicting 3-month outcomes (BSCVA, infiltrate/scar size, and time to epithelialization) using enrollment measures of BSCVA, infiltrate/scar size, baseline epithelial defect, MIC to moxifloxacin, and study arm (steroid vs. placebo) as covariates. Organism was included as a covariate in a separate linear regression model and the overall association with organism species and MIC was assessed with a F-test. If an observation was missing at 3 months, the 3-week observation was carried forward. Reproducibility in the Etest measurement was assessed by the intra-class correlation coefficient (ICC).

Results

Forty-two culture-confirmed bacterial keratitis patients were enrolled in the SCUT pilot study. Thirty-seven organisms were successfully grown from the stored specimens and 34 of these had available outcome data and were analyzed for this study. Five patients with missing outcome data at 3 months had their 3-week results carried forward to 3 months for the purpose of analysis. Among these 34 cases, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were the most common organisms isolated (Table 1). One patient was co-infected with *Haemophilus influenzae* and *S. pneumoniae*, but only the former could be grown from the stored specimen and used in the analysis. The two separate Etest measurements on the same specimen were highly reproducible (ICC= 0.94).

MIC was not associated with logMAR BSCVA at 3 months, (P=0.71, adjusting for enrollment visual acuity and study arm in a linear regression model; Table 2) or time to re-epithelialization (P=0.35, adjusting for enrollment epithelial defect size and study arm in a regression model; Table 3). MIC was associated with infiltrate/scar size at 3 months in a regression model adjusting for enrollment infiltrate/scar size and study arm: every two-fold dilution in MIC level was associated with a 0.33 mm increase in scar size. (P=0.01; Table 4).

We also assessed whether organism species explained the correlation found between MIC and infiltrate/scar size. Organism species was associated with MIC (P<0.0001, F-test), but not with the 3-month infiltrate/scar size (P=0.34, F-test). When organism was added as a categorical variable to the multiple linear regression model predicting 3-month infiltrate/scar size (with enrollment infiltrate/scar size, study arm, and MIC as covariates), the parameter estimate for logMIC was reduced from 0.33 (95% CI 0.08 to 0.59) to 0.22 (95% CI -0.40 to 0.84), decreasing the coefficient by 33%.

Discussion

In this study, we found that antibiotic susceptibility to moxifloxacin is associated with infiltrate/scar size at 3 months in culture-proven bacterial corneal ulcers. Specifically, each 2-fold increase in MIC is associated with a 0.33 mm larger infiltrate/scar size. However, we could not demonstrate an association between MIC and BSCVA or time to epithelialization. One explanation for this finding may be that final visual acuity may be dependent on other factors including pre-existing ocular disease or location of the ulcer. A scar covering the pupil is likely to interfere with visual acuity, while an ulcer away from the pupil may not. Infiltrate/scar size may be a more sensitive measure to determine the effect of MIC. Visual acuity is still a clinically

significant measurement and the correlation between MIC and BSCVA should be further explored with a larger population.

Previous studies have investigated outcomes other than infiltrate/scar size, including complications and ulcer healing times.[13, 14] One report showed that susceptibility to the first antibiotic chosen for treatment was correlated with an increased risk for penetrating keratoplasty.[13] A second study found that cases resistant to ciprofloxacin had a worse cure rate, defined by resolution of inflammation and re-epithelialization.[14] These studies had larger sample sizes and dichotomous predictors and outcomes. Our study had a smaller sample size but used continuous predictor and outcome variables (MIC in μg/ml and infiltrate-scar size in mm, respectively). Continuous variables may result in greater power to detect an association. Also, in contrast to the prior study, our study used a fourth-generation fluoroquinolone, which has come into widespread use for treating ocular infections.

Our findings indicate that MIC and organism species may both play a role in predicting clinical outcome. MIC measures the efficacy of antibiotics against the pathogen, and organism species may provide additional information relevant to clinical outcome such as the intrinsic virulence of the bacteria. We found that organism species is significantly correlated with MIC. This raises the question whether organism species serves as a confounder that actually explains the correlation between MIC and infiltrate/scar size. In our analysis, the coefficient for logMIC decreased from 0.33 to 0.22 (a 33% decrease), suggesting that organism species may partially but not completely confound the observed association. One limitation of this study is that the modest sample size does not afford the power to perform subgroup analyses of the association between MIC and outcome within each organism type. However, this study reveals that MIC provides additional information that predicts clinical outcome beyond knowing the organism.

This study demonstrated that MIC is associated with infiltrate/scar size but not BSCVA or epithelialization time in patients with bacterial keratitis, and that organism species may also play a role in predicting clinical outcomes. To further explore the relationship between MIC, organism species, and clinical outcomes, a study with a larger sample size is necessary. The multi-centered NEI-sponsored SCUT trial (U10EY015114) has a target enrollment of 500 patients and should further our understanding of this subject.

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References

1. Erie JC, Nevitt MP, Hodge DO, Ballard DJ. Incidence of ulcerative keratitis in a defined population from 1950 through 1988. Arch Ophthalmol 1993;111:1665–71. [PubMed: 8155038]
2. Gonzales CA, Srinivasan M, Whitcher JP, Smolin G. Incidence of corneal ulceration in Madurai district, South India. Ophthalmic Epidemiology 1996;3:159–166. [PubMed: 8956320]
3. Whitcher JP, Srinivasan M. Corneal ulceration in the developing world- A silent epidemic. British Journal of Ophthalmology 1997;81:622–23. [PubMed: 9349145]
4. Wilhelmus KR. Review of clinical experience with microbial keratitis associated with contact lenses. Clao J 1987;13:211–4. [PubMed: 3331130]

5. Greenwood D. In vitro veritas? Antimicrobial susceptibility tests and their clinical relevance. J Infect Dis 1981;144:380–5. [PubMed: 7288218]

6. Lorian V, Burns L. Predictive value of susceptibility tests for the outcome of antibacterial therapy. J Antimicrob Chemother 1990;25:175–81. [PubMed: 2318750]

7. Stroman DW, Dajcs JJ, Cupp GA, Schlech BA. In vitro and in vivo potency of moxifloxacin and moxifloxacin ophthalmic solution 0.5%, a new topical fluoroquinolone. Surv Ophthalmol 2005;50 (Suppl 1):S16–31. [PubMed: 16257308]

8. Schlech BA, Alfonso E. Overview of the potency of moxifloxacin ophthalmic solution 0.5% (VIGAMOX). Surv Ophthalmol 2005;50(Suppl 1):S7–15. [PubMed: 16257313]

9. Brown DFJ, Brown L. Evaluation of the Etest, a novel method of quantifying antimicrobial activity. Journal of Antimicrobial Chemotherapy 1991;27:185–190. [PubMed: 2055809]

10. Baker CN, Stocker SA, Culver DH, Thornsberry C. Comparison of the Etest to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. Journal of Clinical Microbiology 1991;29:533–538. [PubMed: 2037671]

11. Performance standards for antimicrobial susceptibility testing. Clinical Laboratory Standards Institute; Wayne, PA: 2006. Performance standards for antimicrobial susceptibility testing.

12. Barron BA, Gee L, Hauck WW, et al. Herpetic Eye Disease Study. A controlled trial of oral acyclovir for herpes simplex stromal keratitis. Ophthalmology 1994;101:1871–82. [PubMed: 7997323]

13. McLeod SD, LaBree LD, Tayyanipour R, Flowers CW, Lee PP, McDonnell PJ. The importance of initial management in the treatment of severe infectious corneal ulcers. Ophthalmology 1995;102:1943–8. [PubMed: 9098300]

14. Wilhelmus KR, Abshire RL, Schlech BA. Influence of fluoroquinolone susceptibility on the therapeutic response of fluoroquinolone-treated bacterial keratitis. Arch Ophthalmol 2003;121:1229–33. [PubMed: 12963604]

Biography

Aiyin Chen is a medical student at the University of California, San Francisco and is planning on pursuing a residency in ophthalmology. She has an interest in international health and implemented the project presented in this paper during a research elective at Aravind Eye Hospital in Madurai, India.

Table 1

Bacterial Organisms Isolated from the Steroids for Corneal Ulcers Trial (n=34)

Organisms	Number (%)	MIC_{50}(μg/ml)	MIC range (μg/ml)
Streptococcus pneumoniae	**16** (47%)	0.18	0.108-0.25
Pseudomonas aeruginosa	**8** (24%)	1.5	1.06-1.73
Viridans-group *Streptococcus* spp	**3** (9%)	0.19	0.108-0.25
Coagulase negative *Staphylococcus*	**3** (9%)	3	0.094-3
Staphylococcus aureus	**2** (6%)	0.05	0.039-0.078
Haemophilus influenzae	**1** (3%)	0.023	0.023
Moraxella spp	**1** (3%)	0.108	0.108
Total	**34 (100%)**		0.023-3

Table 2

Multiple linear regression predicting best spectacle-corrected visual acuity at 3 months in bacterial corneal ulcers (n=34)

Covariate	Coefficient (95% CI)	*P*-value
Enrollment visual acuity (logMAR)	1.08 (0.86, 1.30)	<0.001
Log_2 MIC (μg/ml) to moxifloxacin	0.02 (- 0.07, 0.1)	0.71
Study arm (steroid)	0.17 (- 0.14, 0.47)	0.27

Table 3

Multiple linear regression predicting time to epithelialization in bacterial corneal ulcers (n=34)

Covariate	Coefficient (95% CI)	*P*-value
Log_2 MIC (μg/ml) to moxifloxacin	- 0.49 (- 1.54, 0.55)	0.35
Study arm (steroid)	0.92 (- 2.83, 4.66)	0.62

Table 4

Multiple linear regression predicting infiltrate/scar size at 3 months in bacterial corneal ulcers (n=29)

Covariate	Coefficient (95% CI)	*P*-value
Enrollment infiltrate/scar size (mm)	0.28 (- 0.13, 0.69)	0.17
Log_2 MIC (μg/ml) to moxifloxacin	0.33 (0.08, 0.59)	0.01
Study arm (steroid)	0.25 (- 0.69, 1.18)	0.59