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Fluoroquinolone Treatment and Susceptibility of Isolates From Bacterial Keratitis

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Abstract

Objective—To analyze the relationship between fluoroquinolone use at presentation and minimum inhibitory concentration in bacterial keratitis.

Methods—The Steroids for Corneal Ulcers Trial was a randomized, double-masked, placebo-controlled trial assessing the effect of adjunctive topical corticosteroid treatment on outcomes in bacterial keratitis. After presentation, all patients were treated with moxifloxacin hydrochloride, 0.5%. We compare antibiotic use at presentation with minimum inhibitory concentration against moxifloxacin for all isolates. Separate analyses accounted for organism species and fluoroquinolone generation.

Results—Topical fluoroquinolone use at presentation was reported in 92 of 480 cases (19.2%). Causative organisms in the 480 cases included *Streptococcus pneumoniae* (247 cases [51.5%]), *Pseudomonas aeruginosa* (109 cases [22.7%]), and *Nocardia* species (55 cases [11.5%]). Isolates from patients who reported fluoroquinolone use at presentation had a 2.01-fold–higher minimum inhibitory concentration (95% CI, 1.39-fold to 2.91-fold; $P < .001$). Fourth-generation fluoroquinolones were associated with a 3.48-fold–higher minimum inhibitory concentration than those isolates that were not exposed to pretreatment at enrollment (95% CI, 1.99-fold to 6.06-fold; $P < .001$).

Conclusion—This study provides evidence that prior use of fluoroquinolones is associated with antibiotic resistance.

Aerobic bacterial infection continues to be a major cause of corneal ulceration worldwide.[1] Topical antibiotics are essential in the treatment of bacterial keratitis and are typically successful in clearing the infection. However, strains of bacteria resistant to commonly used ophthalmic antibiotics are of increasing concern, and there is some thought that resistant strains may lead to worse outcomes than susceptible strains.[2–5] Retrospective studies suggest that infectious ocular cases pretreated with topical fluoroquinolones are at higher risk for infection with resistant pathogens.[6, 7] Herein, we use results from a recent randomized controlled trial to compare antibiotic susceptibility of isolates from patients

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using topical fluoroquinolones at presentation with those who were not pretreated with fluoroquinolones to determine whether treatment may result in de novo resistance.[1] The large size of the trial allowed controlling for possible confounding variables such as organism species and type of fluoroquinolone.

METHODS

TRIAL

The Steroids for Corneal Ulcers Trial was a randomized, controlled, double-masked clinical trial with the primary objective to assess the effect of adjunctive topical corticosteroid treatment on outcomes in bacterial keratitis. Briefly, 500 culture-positive cases met enrollment criteria and were randomized to receive either prednisolone phosphate,1% (Bausch & Lomb Pharmaceuticals, Inc) or topical placebo (sodium chloride, 0.9%, and preservative, prepared by Leiter's Compounding Pharmacy).

Enrolled patients had historical and demographic information collected, including prior systemic or topical antibiotic use, type of antibiotic, dose, and duration. Two corneal scrapings were smeared for Gram staining and potassium chloride wet mount. The criterion for a positive bacterial culture was growth of the organism on 1 solid medium at the site of inoculation.[8] Bacterial isolates from the patients enrolled in the trial were tested for susceptibility to moxifloxacin hydrochloride using the Etest (AB BIODISK). All isolates were stored in a −70°C freezer and were subcultured using organism-specific culture media. Etests were repeated on all isolates on a subsequent day, and the geometric mean $\log_2$-transformed minimum inhibitory concentration(MIC) was used for the analysis. The test observer was masked to treatment arm, clinical outcomes, and any prior reading of the MIC. Quality control was performed according to the National Committee for Clinical Laboratory Standards performance standards, recommendations, guidelines, and reports.[9] Isolates with a mixed infection (≥2 distinct organisms isolated) were excluded from the analysis.

STATISTICAL ANALYSIS

Baseline characteristics between the pretreated and unpretreated isolates were compared using Fisher exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Linear regression was performed, predicting $\log_2$-transformed MIC with previous fluoroquinolone treatment as a dichotomous covariate. Sensitivity analyses controlled for fixed effects of organism, age, sex, depth, and duration of symptoms. A second linear model compared MICs in unpretreated isolates vs pretreated isolates grouped by fluoroquinolone generation. Model fit between linear models was assessed using likelihood ratio tests. For presentation in the article, the $\log_2$-transformed MICs were converted back to standard MICs. All analyses were conducted in Stata IC version 10.1 statistical software (StataCorp LP).

ETHICS

University of California, San Francisco, Dartmouth Medical School, and the Aravind Eye Care System all granted institutional review board approval. This study conformed to the tenets of the Declaration of Helsinki, and informed consent was obtained from all subjects.

RESULTS

Of the 500 positive bacterial cultures, 6 had mixed infections, 6 had unidentified bacteria, and 8 had missing MICs, leaving 480 available for analysis. The majority of isolates among the 480 cases were *Streptococcus pneumoniae* (247 cases [51.5%]), followed by *Pseudomonas aeruginosa* (109 cases [22.7%]), and *Nocardia* species (55 cases [11.5%]).[8]

The cases in patients reporting fluoroquinolone use at presentation had a different distribution of organisms than those that were not treated with fluoroquinolones (Table 1) (P <.001). Of the patients with *Nocardia* species, 23 of 55 (41.8%) reported pretreatment at presentation. Similarly, 22 of 109 patients with *P aeruginosa* (20.2%) and 38 of 247 patients with *S pneumoniae* (15.4%) reported using fluoroquinolones prior to enrollment.

A total of 92 patients (19.2%) were pretreated with fluoroquinolones on presentation (Table 1), among whom 50 (54.3%) were male. The median age of the pretreated patients was 50 years (interquartile range, 39–60 years). When comparing them with the 388 patients who were not using fluoroquinolones, there was no significant difference in the number of men (207 [53%]; P = .91) or age (median, 53.5 years; interquartile range, 40–61.5 years; P = .23). One of the 92 pretreated patients (1.1%) had systemic immune or inflammatory disease compared with 23 of 388 (5.9%) who were not using fluoroquinolones prior to enrollment (P = .06).

Bacterial culture isolates from patients reporting fluoroquinolone use at presentation had 2.01-fold–higher MICs (95% CI, 1.39-fold to 2.91-fold; P <.001). Controlling for the organism improved model fit (P <.001), and fluoroquinolone use at presentation remained associated with MIC, showing a 1.38-fold–higher MIC (95% CI, 0.08-fold to 0.84-fold; P = .02) in pretreated isolates. When included as covariates, age, sex, ulcer depth, ulcer location, duration of symptoms, occupation, and systemic immune disease were not significant predictors of MIC and did not markedly change the observed association.

The 92 pretreated patients reported using different fluoroquinolones, including ciprofloxacin hydrochloride (26 patients [28.3%]), ofloxacin (24 patients [26.1%]), gatifloxacin (18 patients [19.6%]), and moxifloxacin (16 patients [17.4%]). The proportions of patients pretreated with second, third, and fourth generations of fluoroquinolones were 57.6%, 4.3%, and 37.0%, respectively (Table 2).[13] Patients who were pretreated with fourth-generation fluoroquinolones (gatifloxacin and moxifloxacin) had 3.48-fold–higher MICs than those who did not report pretreatment at enrollment (95% CI, 1.99-fold to 6.06-fold; P <.001) (Table 3). Only 4 patients reported third-generation fluoroquinolone use (levofloxacin) prior to enrollment, resulting in a nonsignificant increase of 1.68-fold (95% CI, 0.35-fold to 8.11-fold; P = .51). Isolates from patients reporting second-generation fluoroquinolone use (ciprofloxacin, ofloxacin, and norfloxacin) had 1.47-fold–higher MICs than those not reporting fluoroquinolone use, although the results were not statistically significant (95% CI, 0.93-fold to 1.33-fold; P = .10) (Table 3). Adding fluoroquinolone generations as predictors allowed a significant improvement in model fit (P = .004).

COMMENT

Systemic fluoroquinolones select for resistant strains of *P aeruginosa* in bacteremia.[14] Topical fluoroquinolones have been shown to select for resistant conjunctival isolates in both retrospective and prospective studies.[7, 15] In the setting of a large randomized controlled trial, we found that isolates from bacterial ulcers in patients already using a topical fluoroquinolone at presentation at a referral center had twice the MIC of those from patients not reporting fluoroquinolone treatment at presentation.

Patients with fluoroquinolone use at presentation had a different spectrum of bacterial isolates than patients without fluoroquinolone use at presentation (P <.001) (Table 1). Although the relationship between topical fluoroquinolone use at presentation and susceptibility was different in different species, overall we found a significant association between use and MIC when controlling for species. It is not clear whether this can be explained by cases initially infected with resistant strains responding poorly or actual

selection of resistant strains during the course of treatment. Although there were too few cases caused by organisms other than *S pneumoniae, P aeruginosa*, and *Nocardia* species to draw significant conclusions for other organisms, previous surveillance and longitudinal studies have observed emerging resistance in *Staphylococcus* species of ocular flora.[15–18] Results reported here suggest that fluoroquinolone use may select for growth of certain less susceptible species and also selects for resistant strains *within* a species.

Patients who reported using fourth-generation fluoroquinolones had a 3.48-fold–higher MIC than those not using fluoroquinolones. Isolates pretreated with the newer fluoroquinolones appear to be driving the higher MICs in our primary clinical results. This is not surprising because bacterial isolates were tested for susceptibility to moxifloxacin. Analysis of MICs was not performed against other antibiotics in the Steroids for Corneal Ulcers Trial.

Herein, we demonstrate that prior use of fluoroquinolones is associated with antibiotic resistance in bacterial keratitis cases with a positive bacterial culture. Further studies may reveal whether this is de novo or acquired resistance. Regardless, these results suggest that an increase in topical antibiotic therapy before presentation may contribute to increasing observed resistance.

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Table 1

Moxifloxacin Minimum Inhibitory Concentrations of Bacteria Isolates From Patients Using vs Not Using Topical Fluoroquinolones at Presentation

Organism (No. [%])	Fluoroquinolone Use at Presentation	No.	MIC, μg/mL		S, I, or R					
					%			Threshold		
			MIC_{50}	MIC_{90}[a]	S	I	R	S	R	Source
Streptococcus pneumoniae	No	209	0.25	0.38	98	1	1	≤1	≥4	Clinical and Laboratory Standards Institute,[10] 2012
(247 [51.5])	Yes	38	0.25	0.38	97	0	3			
Pseudomonas aeruginosa	No	89	3	8	9	79	12	≤1	>4	Andrews et al,[11] 2007
(109 [22.7])	Yes	20	6	32	5	45	50			
Nocardia species	No	32	1.50	32	41	25	34	≤1	≥4	Andrews et al,[12] 1999
(55 [11.5])	Yes	23	2	32	26	35	39			
Staphylococcus coagulase	No	19	0.25	4	58	11	32	≤0.5	>1	Andrews et al,[11] 2007
negative (21 [4.4])	Yes	2	0.11	NA	100	0	0			
Moraxella species	No	12	0.08	0.13	92	8	0	<0.5	>0.5	Andrews et al,[11] 2007
(13 [2.7])	Yes	1	5.97	NA	0	0	100			
Staphylococcus aureus	No	10	1.03	16	40	20	40	≤0.5	≥2	Clinical and Laboratory Standards Institute,[10] 2012
(11 [2.3])	Yes	1	0.09	NA	100	0	0			
Streptococcus viridans	No	6	0.22	NA	83	0	17	≤1	≥4	Clinical and Laboratory Standards Institute,[10] 2012
group (10 [2.1])	Yes	4	0.22	NA	100	0	0			
Corynebacterium species	No	4	0.22	NA	NA	NA	NA	NA	NA	NA
(4 [0.8])	Yes	0	NA	NA						
Klebsiella species (3 [0.6])	No	3	0.13	NA	NA	NA	NA	NA	NA	NA
	Yes	0	NA	NA						
Pseudomonas species,	No	3	2	NA	NA	NA	NA	NA	NA	NA
non–*P aeruginosa* (3 [0.6])	Yes	0	NA	NA						
Enterobacter species (2	No	1	0.13	NA	100	0	0	≤0.5	>1	Andrews et al,[11] 2007
[0.4])	Yes	1	0.50	NA	100	0	0			
Bacillus species (1 [0.2])	No	0	NA	NA	NA	NA	NA	NA	NA	NA
	Yes	1	19	NA						
Mycobacterium species	No	0	NA	NA	NA	NA	NA	NA	NA	NA

Organism (No. [%])	Fluoroquinolone Use at Presentation	No.	MIC, µg/mL		S, I, or R					
					%			Threshold		
			MIC_{50}	MIC_{90}[a]	S	I	R	S	R	Source
(1 [0.2])	Yes	1	32	NA						
Total (480 [100])	**No**	**388**	**0.25**	**4**	**68**	**22**	**10**	**NA**	**NA**	**NA**
	Yes	**92**	**0.38**	**32**	**57**	**19**	**24**			

Abbreviations: I, intermediate; MIC, minimum inhibitory concentration; NA, not applicable; R, resistant; S, susceptible.

[a]The MIC_{90} requires at least 9 isolates.

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Table 2

Spectrum of Fluoroquinolones

Fluoroquinolone Use at Presentation	No. (%)	MIC_{50} to Moxifloxacin
Second generation	53 (57.6)	0.38
Ciprofloxacin hydrochloride	26 (28.3)	1.29
Ofloxacin	24 (26.1)	0.38
Norfloxacin	3 (3.3)	0.25
Third generation	4 (4.3)	1.10
Levofloxacin	4 (4.3)	1.10
Fourth generation	34 (37.0)	1.75
Gatifloxacin	18 (19.6)	0.38
Moxifloxacin hydrochloride	16 (17.4)	18.99
Type not specified	1 (1.1)	0.38
Total	**92 (100)**	**0.38**

Abbreviation: MIC, minimum inhibitory concentration.

Table 3

Linear Regression Predicting Moxifloxacin Minimum Inhibitory Concentration

Covariate	Fold Increase in MIC	SE	95% CI	*P* Value
Linear Regression Predicting Moxifloxacin MIC (n = 480)				
Fluoroquinolone use at presentation	2.01	1.21	1.39–2.91	<.001
Linear Regression Predicting Moxifloxacin MIC, Controlling for Organism (n = 480)				
Fluoroquinolone use at presentation	1.38	0.19	0.08–0.84	.02
Linear Regression Predicting Moxifloxacin MIC by Fluoroquinolone Generation (n = 480)				
Second-generation fluoroquinolone use	1.47	1.27	0.93–1.33	.10
Third-generation fluoroquinolone use	1.68	2.22	0.35–8.11	.51
Fourth-generation fluoroquinolone use	3.48	1.33	1.99–6.06	<.001

Abbreviation: MIC, minimum inhibitory concentration.