Penetration of Ofloxacin and Ciprofloxacin Into the Aqueous Humor of Eyes With Functioning Filtering Blebs

A Randomized Trial

Louis B. Cantor, MD; Eric Donnenfeld, MD; L. Jay Katz, MD; Winnie L. Gee, MS; Charles D. Finley, MD; Vipul K. Lakhani, MD; Joni Hoop, CCRC; Kelly Flarty, MS

Objective: To determine concentrations of ofloxacin and ciprofloxacin hydrochloride in aqueous humor after topical or combined topical and oral administration in eyes with filtering blebs.

Design: A prospective, investigator-masked, randomized, controlled comparative study involving 36 eyes of 34 patients with functioning filtering blebs who were to undergo cataract surgery. Treatment groups received either topical ofloxacin or topical ciprofloxacin (instillation of 0.3% ophthalmic solution every 30 minutes for 4 hours before surgery), or a combined topical plus oral regimen (ciprofloxacin hydrochloride, four 100-mg tablets, or ofloxacin, one 400-mg tablet, administered 24-26, 12-14, and 2 hours preceding surgery). The main outcome measure was antibiotic concentration measured by chromatographic separation and mass spectrometry of aqueous samples obtained during surgery.

Results: Topical antibiotic treatment yielded mean concentrations of ofloxacin, 0.75 µg/mL, and ciprofloxacin, 0.21 µg/mL, in aqueous. With combined topical and oral therapy, significantly more ofloxacin was measured than ciprofloxacin (3.84 µg/mL vs 0.35 µg/mL [$P<.001$]). The combination regimen produced significantly greater ofloxacin levels than did topical therapy alone ($P=.007$).

Conclusions: Ofloxacin penetrates better than ciprofloxacin into the aqueous of eyes with filtering blebs, particularly after combined topical and oral administration, by which ofloxacin reaches more than a 10-fold greater concentration than does ciprofloxacin. Combined topical and oral therapy with ofloxacin may be beneficial in the treatment of bleb-associated infections.

Arch Ophthalmol. 2001;119:1254-1257

From the Department of Ophthalmology, Indiana University School of Medicine, Indianapolis (Drs Cantor, Finley, and Lakhani, and Ms Hoop); Department of External Disease/Cornea, Manhattan Eye, Ear and Throat Hospital (Dr Donnenfeld), and Cornea Department, North Shore University Hospital (Dr Donnenfeld), New York, NY; Wills Eye Hospital (Dr Katz and Ms Flarty) and Department of Ophthalmology, Jefferson Medical College (Dr Katz), Philadelphia, Pa; and the Department of Biopharmaceutical Sciences, University of California, San Francisco (Ms Gee).
The authors have no financial interest in the drugs used in this study.

THE PRIMARY organisms involved in bleb-associated infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* species, and *Haemophilus infuenzae*.[1] Conventional medical treatment of bleb infections typically has used vancomycin and cefazolin, both of which have poor aqueous penetration and are not available in a commercial form for topical administration. In contrast, fluoroquinolones such as ofloxacin and ciprofloxacin are commercially available for topical administration. Fluoroquinolones are highly soluble and have a broad spectrum of activity. They offer the potential for broad coverage against infection if sufficient quantities of antibiotic penetrate the eye to achieve high levels in aqueous humor. Moreover, fluoroquinolones can be given as an oral agent on an outpatient basis. Previous work[2] suggests that combining oral and topical administration of fluoroquinolones may greatly increase the aqueous concentration of these drugs.

These properties make fluoroquinolones attractive candidates for treatment of ocular infections. The purpose of this study was to evaluate the potential usefulness of the fluoroquinolones ofloxacin and ciprofloxacin for the treatment of bleb-associated infections. Because antibiotics achieving the highest concentrations in aqueous have the greatest potential for the management of infection in eyes that have undergone glaucoma filtration surgery, we sought to determine whether ofloxacin or ciprofloxacin penetrated better into eyes with filtering blebs and whether a combined topical plus oral treatment regimen produced higher antibiotic concentrations in aqueous than did topical administration alone.

RESULTS

Topical administration alone yielded antibiotic concentration (mean±SD) in aqueous humor of 0.75±0.48-µg/mL ofloxacin compared with 0.21±0.20-

METHODS

STUDY POPULATION

The study involved 36 eyes with functioning filtering blebs from 34 patients who were to undergo cataract surgery by 3 of us (L.B.C., E.D., and L.J.K.) at our respective hospitals. Patients who were scheduled to have cataract surgery in an eye with a functioning filtering bleb were enrolled in the study. Key exclusion criteria included the following: an ongoing ocular inflammatory disease, bleb leakage, any suspected infection, and known contraindications to use of the study medications. Enrolled patients ranged in age from 37 to 86 years and included 18 men and 16 women. All aspects of the study complied with the Declaration of Helsinki, and protocol and informed consent forms were reviewed and approved by an institutional review board.

MASKING, INTERVENTION, AND TIMING

Thirty-six eyes were randomly assigned to 1 of 4 treatment groups of 8 or 10 eyes each (**Figure 1**). Treatment group assignment was determined by random allocation of shuffled 3×5-inch index cards, which were kept at Indiana University, Indianapolis. The code for the treatment group assignments was forwarded with the aqueous humor specimens to the University of California, San Francisco, laboratories, where the analysis for antibiotic levels was performed. Treatment groups received either topical ofloxacin, topical and oral ofloxacin, topical ciprofloxacin, or topical and oral ciprofloxacin. The surgeon investigators were masked with regard to the antibiotic regimen received by the patients, and aqueous samples were identified by coded labels to mask the investigators involved in analysis of the samples. Topical doses of ofloxacin (Ocuflox; Allergan, Inc, Irvine, Calif) or ciprofloxacin hydrochloride (Ciloxan; Alcon Laboratories, Inc, Fort Worth, Tex) were administered by instillation of 1 drop of a 0.3% ophthalmic solution every half hour, beginning 4 hours before surgery, with the last dose administered 30 minutes before surgery. Oral doses of ofloxacin (Floxin, one 400-mg tablet; Ortho-McNeil Pharmaceutical, Raritan, NJ) and ciprofloxacin hydrochloride (Cipro, four 100-mg tablets; Bayer Corporation, West Haven, Conn) were administered 24 to 26, 12 to 14, and 2 hours before surgery.

Preoperative evaluation was performed in the usual manner for eyes being considered for cataract surgery. However, special attention was given to the appearance of the filtering bleb, and its size and vascularity were noted. In addition, a Seidel test was used to detect any bleb or transconjunctival leakage. Eyes with bleb leaks were excluded.

Aqueous samples (0.1-0.2 mL) were withdrawn during surgery, stored at –80°C, and sent to the University of California, San Francisco, for analysis.

DRUG CONCENTRATIONS

The antibiotic content of all aqueous samples was measured following chromatographic separation and mass spectrometric detection using a protocol adapted from Ren et al[3] and validated for use with human aqueous humor samples.[4] Aqueous humor samples (10 µL) were diluted with a combination of 20 µL of acetonitrile, 50 µL of triamterene internal standard solution, and 150 µL of 45% acetonitrile. Aliquots (20 µL) were then separated by high-performance liquid chromatography (PE Sciex-API III System; PE Sciex, Concord, Ontario) equipped with a butyl column (4.6×50.0 mm, 5-µm particle size) using a combination of 50% acetonitrile, 0.06% trifluoroacetic acid, 5mM ammonium acetate, and 0.0006% ammonium phosphate as the mobile phase. Mass spectrometric detection with sample inlet by heated nebulizer, positive ionization by atmospheric pressure chemical ionization, and mass scanning by multiple reaction monitoring were used. Antibiotic concentrations in samples were calculated with the use of standard curves that were prepared by spiking rabbit aqueous humor with a constant amount of internal standard and known amounts of ofloxacin or ciprofloxacin. Rabbit aqueous humor was used in the standard curves because of the unavailability of human aqueous humor. The limit of detection for both ofloxacin and ciprofloxacin was 0.01 µg/mL.

STATISTICAL ANALYSIS

Antibiotic concentrations were analyzed with the use of descriptive statistics (mean±SD). Three samples from ciprofloxacin-treated patients contained no quantifiable antibiotic; these were assigned a value of 0.0099 µg/mL. Results were similar when a value of 0 µg/mL was assigned to these samples. Data from each eye were analyzed separately for the 2 patients who had both eyes included in the study. A 2-way analysis of variance was used to test for the effects of type of antibiotic and drug regimen on antibiotic concentrations in aqueous. The *t* test was used for post hoc comparisons between individual treatment groups. Differences were considered statistically significant at $P<.05$.

µg/mL ciprofloxacin, a significant difference ($P=.005$; **Figure 2**). Combined topical plus oral administration yielded antibiotic concentrations in aqueous of 3.84 ±2.72-µg/mL ofloxacin compared with 0.35±0.30-µg/mL ciprofloxacin. Again, ofloxacin concentrations were significantly higher than were ciprofloxacin concentrations ($P<.001$). We also compared antibiotic concentrations produced by topical vs combined topical and oral administration for each drug. For ofloxacin, the combined dosing regimen of topical and oral administration produced greater antibiotic concentration in aqueous than did topical instillation alone ($P=.007$). However, ciprofloxacin concentrations in aqueous were not significantly enhanced by the addition of oral dosing to topical administration ($P=.14$).

COMMENT

The risk of bleb infection and subsequent vision-threatening endophthalmitis in eyes with antimetabolite filtering blebs may be as high as 1% per year.[5] Although infection can be a major health risk in postoperative patients with glaucoma, few studies have addressed ocular drug penetration for treatment of these conditions in pa-

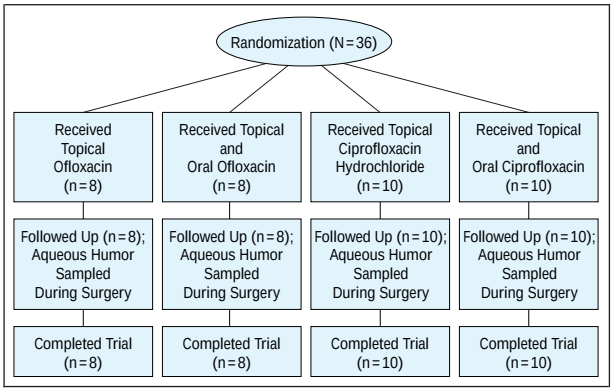

Figure 1. Flow diagram of the study randomization and procedure. No patients withdrew from the study.

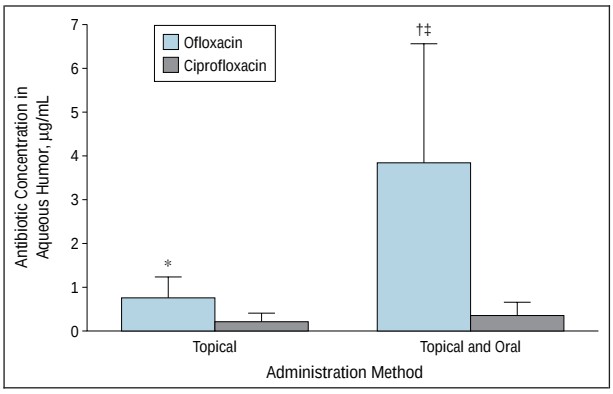

Figure 2. Antibiotic concentrations in aqueous humor of eyes with filtering blebs after different dosing regimens. Eyes received 0.3% antibiotic topically every 30 minutes for 4 hours preceding surgery. Some patients also received oral antibiotic, 1200 mg, in divided doses in the 26 hours before surgery. Values are given as mean±SD (n=8 or 10 for each group). Asterisk indicates $P=.005$ vs ciprofloxacin; dagger, $P<.001$ vs ciprofloxacin; and double dagger, $P=.007$ vs topical administration.

tients with filtering blebs. This study shows that ofloxacin is found at significantly greater concentration than ciprofloxacin in the aqueous humor of eyes with functioning filtering blebs after cataract surgery, whether administered topically or as a combined regimen of topical and oral administration. Ofloxacin was 3.5-fold more concentrated in aqueous than was ciprofloxacin when applied topically and 10-fold more concentrated when oral therapy was added to the topical therapy. These differences may have clinical significance, particularly for the combined therapy of topical and oral administration, because ofloxacin levels well exceeded the MIC_{90} (minimum inhibitory concentration required to inhibit growth of 90% of the bacterial isolates tested) for *S aureus* and *S epidermidis*, whereas ciprofloxacin levels were less than the MIC_{90} for these organisms.[6] This may make ofloxacin a prudent choice for the management of bleb-associated infections.

Antibiotic concentrations measured in this study are in agreement with those of previous researchers who measured aqueous levels of fluoroquinolones applied either as topical drops[6-8] or as collagen shields.[9] However, patients in the earlier studies did not have a history of trabeculectomy. Fluoroquinolone concentrations in the aqueous are similar in patients with or without trabeculectomy, which suggests that the presence of the filtering bleb (in the absence of corneal and conjunctival leakage) does not affect antibiotic penetration.

Minimum Inhibitory Concentrations (MIC_{90}) of Ofloxacin Against Selected Ocular Pathogens In Vitro*

Bacterial Pathogen	Ofloxacin MIC_{90}, µg/mL	Source
Escherichia coli	0.125	12, 13
Haemophilus influenzae	0.03	12, 13
Proteus mirabilis	0.125	12, 13
Pseudomonas aeruginosa	4	12
Staphylococcus aureus	0.5	12
Staphylococcus epidermidis	0.5	12
Streptococcus pneumoniae	2	12

*MIC_{90} indicates the minimum inhibitory concentration required to inhibit the growth of 90% of the bacterial isolates tested.

An important result of our study is that treatment with a combination of topical and oral ofloxacin resulted in a more than 5-fold greater ofloxacin concentration than did treatment with topical drug alone. Bleb-associated endophthalmitis is often caused by *S epidermidis* or by gram-negative bacteria that are sensitive to ofloxacin,[10] and oral dosing has been demonstrated to result in concentrations of ofloxacin in aqueous humor sufficient for activity against some of these organisms.[11] Furthermore, both oral and topical ofloxacin have been shown to penetrate the vitreous.[2] This penetration of antibiotic to the posterior eye may reduce the risk of bleb infection progressing to endophthalmitis. Ofloxacin levels seen in this study with combined oral and topical treatment exceed the MIC_{90} of common gram-positive ocular pathogens, including *S aureus*, *S epidermidis*, and *Streptococcus pneumoniae*. In addition, these ofloxacin levels were in a range similar to the MIC_{90} of the problematic gram-negative *Pseudomonas aeruginosa* and well exceeded the MIC_{90} of other gram-negative pathogens like *H influenzae*, *Proteus mirabilis*, and *Escherichia coli* (**Table**). A combined topical and oral dosing regimen of ofloxacin, therefore, may have great potential for effective treatment of blebitis and bleb-related endophthalmitis after glaucoma filtration surgery.[14] Further studies are needed to explore these possibilities.

Accepted for publication May 10, 2001.

This study was supported by an unrestricted grant from Research to Prevent Blindness, Inc, New York, NY, and by a grant from the Glaucoma Research and Education Foundation, Indianapolis.

Presented in part as a poster at the 103rd Annual Meeting of the American Academy of Ophthalmology and the XXII Congress of the Pan-American Association of Ophthalmology, held jointly in Orlando, Fla, October 24-27, 1999.

Corresponding author and reprints: Louis B. Cantor, MD, Department of Ophthalmology, Indiana University School of Medicine, 702 Rotary Cir, Room 141, Indianapolis, IN 46202 (e-mail: lcantor@iupui.edu).

REFERENCES

1. Waheed S, Ritterband DC, Greenfield DS, Liebmann JM, Seedor JA, Ritch R. New patterns of infecting organisms in late bleb-related endophthalmitis: a ten year review. *Eye.* 1998;12:910-915.
2. Donnenfeld ED, Perry HD, Snyder RW, Moadel K, Elsky M, Jones H. Intracorneal, aqueous humor, and vitreous humor penetration of topical and oral ofloxacin. *Arch Ophthalmol.* 1997;115:173-176.
3. Ren Q, Li X, Horng H, Shi R. Quantitation of ketorolac and ofloxacin in rabbit plasma by LC/MS/MS. Poster presented at: annual meeting of the American Association of Pharmaceutical Scientists (AAPS); October 29–November 2, 2000; Indianapolis, Ind.
4. Ren Q, Li X, Horng H, Shi R. Quantitation of ketorolac and ofloxacin in rabbit plasma by LC/MS/MS [abstract]. In: Program and abstracts of the annual meeting of the American Association of Pharmaceutical Scientists (AAPS); October 29–November 2, 2000; Indianapolis, Ind. Available at: http://www.aapspharmaceutica.com/scientificjournals/pharmsci/am_abstracts/2000/2215.html. Accessed July 6, 2001.
5. Greenfield DS, Suner IJ, Miller MP, Kangas TA, Palmberg PF, Flynn HW Jr. Endophthalmitis after filtering surgery with mitomycin. *Arch Ophthalmol.* 1996; 114:943-949.
6. Beck R, van Keyserlingk J, Fischer U, Guthoff R, Drewelow B. Penetration of ciprofloxacin, norfloxacin and ofloxacin into the aqueous humor using different topical application modes. *Graefes Arch Clin Exp Ophthalmol.* 1999;237:89-92.
7. Cekic O, Batman C, Totan Y, et al. Penetration of ofloxacin and ciprofloxacin in aqueous humor after topical administration. *Ophthalmic Surg Lasers.* 1999;30: 465-468.
8. von Keyserlingk J, Beck R, Fischer U, Hehl EM, Guthoff R, Drewelow B. Penetration of ciprofloxacin, norfloxacin and ofloxacin into the aqueous humour of patients by different topical application routes. *Eur J Clin Pharmacol.* 1997;53: 251-255.
9. Taravella MJ, Balentine J, Young DA, Stepp P. Collagen shield delivery of ofloxacin to the human eye. *J Cataract Refract Surg.* 1999;25:562-565.
10. Ciulla TA, Beck AD, Topping TM, Baker AS. Blebitis, early endophthalmitis, and late endophthalmitis after glaucoma-filtering surgery. *Ophthalmology.* 1997;104: 986-995.
11. Hanioglu-Kargi S, Basci N, Soysal H, Bozkurt A, Gursel E, Kayaalp O. The penetration of ofloxacin into human aqueous humor given by various routes. *Eur J Ophthalmol.* 1998;8:33-36.
12. Osato MS, Jensen HG, Trousdale MD, et al. The comparative in vitro activity of ofloxacin and selected ophthalmic antimicrobial agents against ocular bacterial isolates [published correction appears in *Am J Ophthalmol.* 1991;112:478-479]. *Am J Ophthalmol.* 1989;108:380-386.
13. Wiedemann B, Grimm H. Susceptibility to antibiotics: species incidence and trends. In: Lorian VL, ed. *Antibiotics in Laboratory Medicine.* 4th ed. Baltimore, Md: Williams & Wilkins; 1996:900-1168.
14. Ayyala RS, Bellows AR, Thomas JV, Hutchinson BT. Bleb infections: clinically different courses of "blebitis" and endopthalmitis. *J Ophthalmic Nurs Technol.* 1997;16:292-300.

From the Archives of the ARCHIVES

A look at the past . . .

Septic Embolism of the Retina Following Extraction of a Carious Tooth

A patient 40 years old had a tooth extracted. Immediately afterwards a high septic fever set in. Three days later the patient noticed loss of vision, and there was to be seen in the region of the macula a large, elevated white patch with a network of little retinal vessels at its margin and some hemorrhages on its surface.

Reference: Foster M. Progress of ophthalmology. *Arch Ophthalmol*. 1925;54:104.