ABSTRACT

An in vivo prospective study compared the effectiveness of 0.3% ciprofloxacin to 0.3% ofloxacin against Pseudomonas aeruginosa *keratitis in rabbits. Ofloxacin-treated corneas yielded an average amount of colony-forming units (CFUs) of* P aeruginosa *that was statistically significantly higher than that of ciprofloxacin-treated corneas ($4.7 \times 10^4 \pm 2.2 \times 10^3$ vs $2.5 \times 10^3 \pm 1.0 \times 10^2$). Thus, ciprofloxacin was more effective than ofloxacin in the early reduction of CFUs in* P aeruginosa *keratitis in rabbits.*

ORIGINAL ARTICLE

Topical 0.3% Ciprofloxacin vs Topical 0.3% Ofloxacin in Early Treatment of *Pseudomonas aeruginosa* Keratitis in a Rabbit Model

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The initial treatment of bacterial keratitis consists of broad-spectrum antibiotic therapy.[1] This is necessary because the Gram stain is often negative even in culture-positive bacterial keratitis.[2] The traditional regimen for the initial therapy for bacterial keratitis consisted of a fortified aminoglycoside to cover gram-negative organisms and a formulated first-generation cephalosporin or vancomycin to cover gram-positive organisms.[3] Unfortunately, there are many disadvantages to formulated antibiotic drops. They are difficult to obtain, there may be variations in pH and concentration, and they have a limited shelf life.[4] In addition, fortified drops have substantial corneal toxicity.[5] Therefore, a commercially available, potent, broad-spectrum topical antibiotic that could be used as monotherapy to treat presumed bacterial keratitis would be beneficial.

The fluoroquinolone family of antibiotics is structurally related to nalidixic acid.[6] They work by inhibiting bacterial DNA gyrase, resulting in bacterial cell death.[6] They have a broad spectrum of antimicrobial activity with the exception of anaerobic bacteria.[7,8] They also have minimal corneal toxicity[9] and produce bacterial resistance at a lower rate than do many other antibiotics.[10] The 3 fluoroquinolones approved for use

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in ophthalmology are ciprofloxacin, norfloxacin, and ofloxacin.[11] Both ciprofloxacin and ofloxacin are more potent than norfloxacin against many organisms, including *Pseudomonas aeruginosa*.[12] Ciprofloxacin and ofloxacin, but not norfloxacin, are approved by the Food and Drug Administration (FDA) to treat bacterial keratitis.[11]

The choice of which antibiotic to use in a particular clinical situation has important therapeutic consequences. In the case of bacterial keratitis, more corneal destruction and severe corneal scarring may result from the use of an inappropriate antibiotic. This is especially true in the case of *P aeruginosa* keratitis since *P aeruginosa* elaborates a calcium-activated protease, which leads to liquefaction necrosis of the corneal stroma.[13,14] Corneal perforation and/or extensive corneal scarring may result. Early treatment with appropriate antimicrobial therapy may limit the extent of corneal destruction and help to prevent severe sequelae from occurring.

Either ciprofloxacin or ofloxacin is effective as monotherapy to treat bacterial keratitis.[15] Each drug has been shown in prospective studies to be as effective as conventional therapy with regard to time to epithelial healing and total treatment time required.[9,16-18] To our knowledge, no studies in the literature directly compare these 2 antibiotics in an in vivo model of bacterial keratitis. However, there is in vitro evidence suggesting that ciprofloxacin is a more potent antibiotic against many types of bacteria, including *P aeruginosa*.[19,20]

There are several in vitro methods to determine the relative effectiveness of different antibiotics in a given clinical setting. These include the sensitivity of the organism to the antibiotic, the concentration of the antibiotic at the site of infection, and pharmacodynamic measures of antibiotic effectiveness such as kill curves.[19] Antibiotic sensitivity usually is measured by the minimum inhibitory concentration, which is a quantitative measure of the minimum concentration of antibiotic that inhibits the growth of 90% of the strains of a particular organism.[21] The concentration of antibiotics in ocular tissues can be determined using a variety of experimental techniques. The measurements obtained depend on many factors, such as the dosing regimen of the antibiotic, the absence or presence of a corneal epithelial defect, the absence or presence of ocular inflammation, the type of assay used, and the use of a human or animal model.[22] A ratio called the inhibitory quotient integrates the minimum inhibitory concentration and the concentration of the antibiotic at the site of infection in an attempt to compare different antibiotics given data regarding their potency and penetration.[23] A pharmacodynamic measure such as a kill curve determines the rate at which a given antibiotic reduces colony counts of a particular bacterial culture.[19]

These in vitro measures can be used to compare ciprofloxacin with ofloxacin in the treatment of *P aeruginosa* keratitis. The minimum inhibitory concentration of ciprofloxacin against *P aeruginosa* is 4 to 8 times lower than for ofloxacin, indicating that ciprofloxacin is a more potent antibiotic than ofloxacin against this particular organism.[20] Studies looking at the relative penetration of these antibiotics show variable results, with ofloxacin showing enhanced penetration relative to ciprofloxacin in most studies.[20,22,24,25] Kill curve data show that ciprofloxacin reduces colony counts of *P aeruginosa* at a faster rate than does ofloxacin.[19]

The purpose of this study was to determine whether the previously described in vitro determinations that appear to favor ciprofloxacin over ofloxacin in the treatment of *P aeruginosa* keratitis are supported by the results of an in vivo model of *P aeruginosa* keratitis in rabbits. Because in vitro kill curve data indicate that more rapid bacterial killing occurs with ciprofloxacin vs ofloxacin, we compared these 2 fluoroquinolone antibiotics in the early treatment of *P aeruginosa* keratitis in a rabbit model.

Materials & Methods

The animals used in this study were maintained in animal care facilities accredited by the American Association of Laboratory Animal Science, and the procedures performed were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty female New Zealand albino rabbits (weighing 3.0 to 4.0 kg each) were used in this study, and an additional 6 rabbits were used in pilot studies. Anesthesia was induced using an intramuscular injection of ketamine and xylazine hydrochloride mixture and topical proparacaine hydrochloride. The surface of the cornea was marked using a 3-mm corneal trephine, and mechanical debridement of the corneal epithelium within this area was performed using a No. 15 Beaver blade. A Hamilton microsyringe (Hamilton Company, Reno, Nev) was used to perform an intrastromal injection of 40 µL of *P aeruginosa* in its logarithmic growth phase (strain ATCC No. 27853 in a suspension of 2.0 McFarland units; Fig 1). The minimum inhibitory concentration against this strain of *P aeruginosa* is 0.5 µg/mL for ciprofloxacin and 2.0 µg/mL for ofloxacin. We waited 12 hours after injection of organisms into the corneal stroma before beginning topical antibiotic treatment (Fig 2).

The rabbits were divided into 3 groups. Group A contained 9 rabbits (18 eyes) and received study drug A. Group B contained 9 rabbits (18 eyes) and received study drug B. Group C, the control group, contained 2 rabbits (4 eyes) and received no treatment. The antibiotics used were commercially available 0.3% ciprofloxacin (Ciloxan, Alcon Laboratories, Fort Worth, Tex) and 0.3% ofloxacin (Ocuflox, Allergan Inc, Irvine, Calif) in coded bottles. The investigators were masked as to the identity of the bottles. Treated eyes received 1 drop of study drug every hour for 3 hours, beginning 12 hours after inoculation of the cornea with *P aeruginosa*.

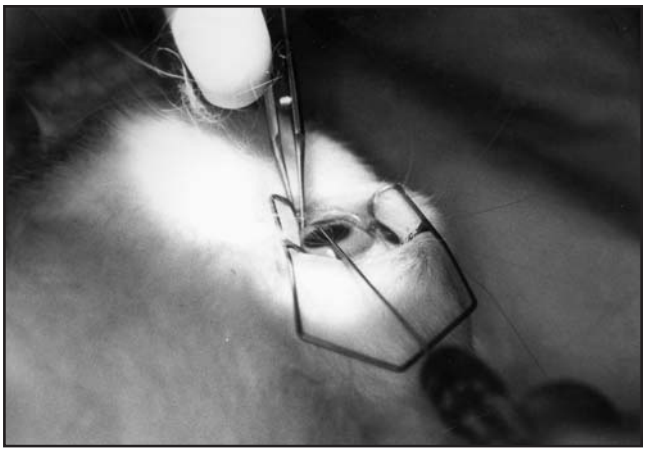

Fig 1.—Intrastromal injection of *Pseudomonas aeruginosa* into a rabbit cornea after creation of a 3-mm epithelial defect.

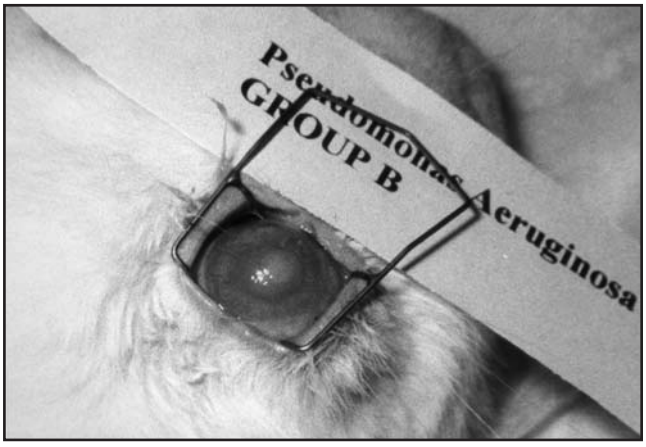

Fig 2.—*Pseudomonas aeruginosa* corneal ulcer after 12 hours of incubation.

The animals were then killed 1 hour after the last dose using an intravenous lethal dose of sodium pentobarbital after induction of anesthesia with ketamine and xylazine. An 8-mm trephine was used to mark the corneas of all eyes. The corneal buttons were excised using a corneal scleral scissors. These buttons were placed in sterile Petri dishes for transport to the hospital microbiology laboratory. The Petri dish covers were removed, and a sterile surgical razor was used to chop each cornea into small pieces. One milliliter of nonbacteriostatic saline was added to each Petri dish to arrive at a starting concentration of 10^{-2}. The resulting slurry was placed in an 80-mL bag (Seaward Stomacher, Fisher Scientific Company, Jessup, Md) and homogenized for 2 minutes. Serial dilutions were carried out to 10^{-5} using nonbacteriostatic saline and plated onto trypticase soy broth with agar with 5% sheep blood agar and MacConkey agar plates. The cultures were allowed to incubate in 5% CO_2 at 35°C in an incubator for 24 hours.

Colony counts were performed at 24 hours on plates that had between 1 and 200 colonies and were multiplied by the corresponding dilution. Photographs were taken of all culture plates (Fig 3). The number of colony-forming units on the countable plates was averaged for the blood agar and the MacConkey agar plates individually. These values were then averaged together to calculate the average number of colony-

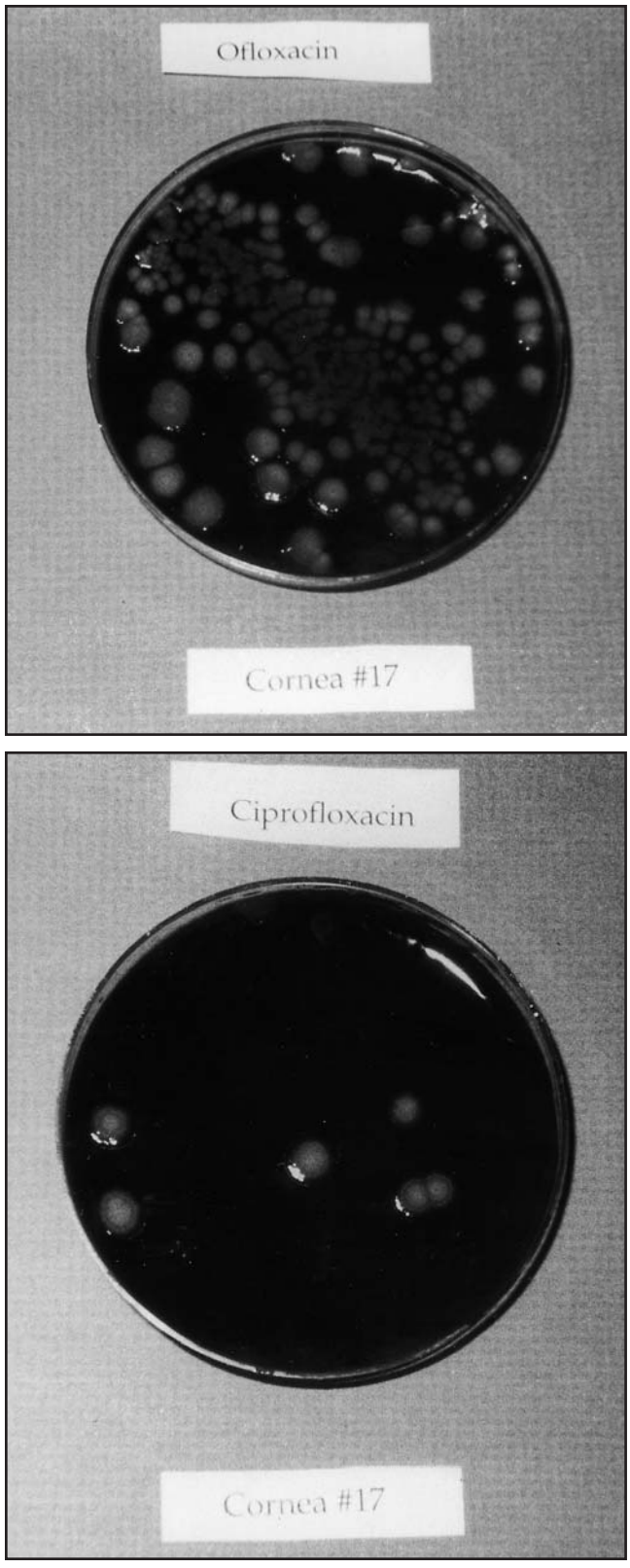

Fig 3.—Representative culture plates (serial dilution 10^{-2}) showing the number of colony-forming units in a cornea from the ofloxacin-treated group (cornea 17A, top) and from the ciprofloxacin-treated group (cornea 17B, bottom).

forming units for each cornea. The minimum inhibitory concentration for the test organism was determined before beginning the study and from organisms isolated from culture plates after the study from each group. The minimum inhibitory concentrations of ciprofloxacin and ofloxacin to the test organism were the same before and after the study.

We determined the dosing regimen used in this study by first performing 3 pilot studies using the techniques previously described. In the first pilot study, 2 eyes were treated with 1 drop of 0.3% ciprofloxacin every half hour for 6 hours and 2 eyes were treated with 1 drop of 0.3% ofloxacin every half hour for 6 hours. This resulted in sterilization of all 4 corneas. In the second pilot study, 2 eyes were treated with a single drop of 0.3% ciprofloxacin and 2 eyes were treated with a single drop of 0.3% ofloxacin. This dosing regimen resulted in colony-forming units too numerous to count for all 4 corneas. In the third pilot study, 2 eyes were treated with 1 drop of 0.3% ciprofloxacin every hour for 3 hours and 2 eyes were treated with 1 drop of 0.3% ofloxacin every hour for 3 hours. This resulted in countable colony-forming units for each of the 4 corneas and was the dosing regimen used for our actual study.

Comparison between the treatment groups was performed using the 2-tailed Student *t* test for unpaired samples, with a *P* value of ≤ .05 considered significant.

Results

The bacterial colony-forming unit counts are shown for each group in Table 1. After 3 hours of treatment, the mean colony count in the control group was $1.3 \times 10^6 \pm 6.9 \times 10^5$. Group A (0.3% ciprofloxacin) had $2.5 \times 10^3 \pm 1.0 \times 10^2$ colony-forming units and group B (0.3% ofloxacin) had $4.7 \times 10^4 \pm 2.2 \times 10^3$ colony-forming units.

Using a 2-tailed Student *t* test, there was a statistically significant reduction of colony counts in group A (ciprofloxacin) compared with the control group ($P < .001$). There also was a statistically significant reduction of colony counts in group B (ofloxacin) compared to the control group ($P < .001$). Treatment with ciprofloxacin resulted in a greater reduction of colony-forming units than did treatment with ofloxacin ($P = .021$; Fig 4).

Discussion

Pseudomonas aeruginosa keratitis is a potentially sight-threatening condition due to the rapid tissue destruction and liquefaction necrosis of the cornea that commonly occurs.[13] The calcium-activated protease released by *P aeruginosa* is responsible for this effect. Because corneal perforation and severe corneal scarring can result from this infection, prompt treatment is required.

The conventional treatment of *P aeruginosa* keratitis has consisted of a fortified aminoglycoside, possibly combined with a semisynthetic penicillin such as ticarcillin or carbenicillin.[10] These drops are extremely toxic to the corneal epithelium,[5] have a limited shelf life, and can be difficult to obtain; also, increasing bacterial resistance to these antibiotics has been described.[26,27]

The FDA has approved both 0.3% ciprofloxacin and 0.3% ofloxacin to treat bacterial keratitis.[11] These fluoroquinolone antibiotics have a broad spectrum of activity against gram-positive and gram-negative organisms, including *P aeruginosa*.[7,8] They have been

TABLE 1

Average Number of Colony-Forming Units (CFU) for Each Cornea

	Average CFU by Group		
Cornea	Ciprofloxacin (Group A)	Ofloxacin (Group B)	Control
1	900	10,033	660,000
2	2,150	0	810,000
3	100	7,050	1,650,000
4	3,400	350	2,100,000
5	1,050	131,250	
6	900	44,767	
7	400	4,600	
8	1,500	236,500	
9	4,500	22,063	
10	1,300	6,225	
11	2,050	11,067	
12	2,500	25,250	
13	300	9,033	
14	5,200	6,825	
15	400	12,083	
16	1,100	54,475	
17	1,700	255,333	
18	16,267	14,717	
Average	2,540 ± 103	47,312 ± 2,183	1,305,000 ± 6.9×10^5

Note: The control group had only 4 eyes.

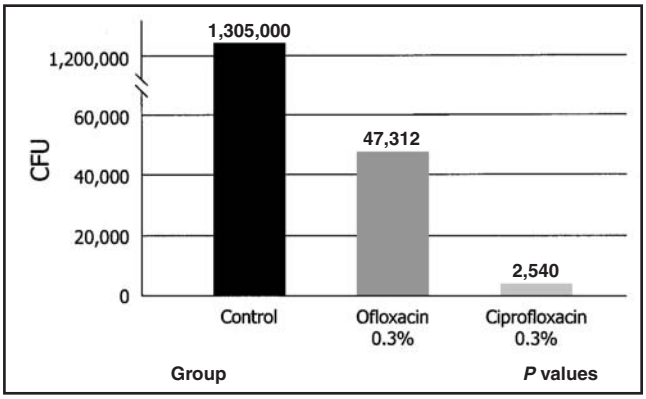

Fig 4.—Average number of colony-forming units (CFU) in corneas from the control group, from treatment group A (0.3% ofloxacin), and from treatment group B (0.3% ciprofloxacin).

shown in animal studies and in clinical trials to be effective against *P aeruginosa* keratitis.[17,18]

Data on antibiotic potency, as measured by minimum inhibitory concentration, indicate that *P aeruginosa* is more sensitive to ciprofloxacin than to ofloxacin. However, most studies show that the corneal concentration of ofloxacin is somewhat higher than ciprofloxacin with equivalent dosing.[23] The inhibitory quotient, which integrates antibiotic potency and antibiotic tissue concentration, favors ciprofloxacin over ofloxacin in the treatment of *P aeruginosa* keratitis.[20] In addition, pharmacodynamic data using kill curves indicate that ciprofloxacin reduces the colony-forming units of *P aeruginosa* more rapidly than does ofloxacin.[19]

Our study used a previously described model of *P aeruginosa* keratitis in rabbits[28] to determine whether the in vitro difference in the fluoroquinolones described above is also observed in an in vivo model.[29] In pilot studies, we found that both antibiotics, given enough time, were able to sterilize the infected corneas. However, early in the course of treatment the reduction of colony-forming units was statistically greater with 0.3% ciprofloxacin than with 0.3% ofloxacin. This early reduction of colony-forming units may decrease corneal tissue destruction resulting from release of bacterial proteases.[13]

Our in vivo study shows that 0.3% ciprofloxacin is more effective than 0.3% ofloxacin in reducing colony-forming units in the early treatment of *P aeruginosa* keratitis in rabbits. Thus, both in vitro and in vivo data suggest that 0.3% ciprofloxacin may be more effective than 0.3% ofloxacin in the treatment of *P aeruginosa* keratitis.

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