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REVIEW

Review of Third- and Fourth-Generation Fluoroquinolones in Ophthalmology: In-Vitro and In-Vivo Efficacy

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ABSTRACT

***Introduction*:** Beginning with second-generation ciprofloxacin 0.3% and ofloxacin 0.3%, fluoroquinolones have been widely used in the treatment and prophylaxis of ocular infections. However, their in-vitro potencies have been decreasing steadily since their introduction. Third-generation levofloxacin 0.5% produces higher ocular tissue penetration, thereby reducing the risk of selecting for decreased fluoroquinolone potency. Fourth-generation gatifloxacin 0.3% and moxifloxacin 0.5% have structural modifications that both reduce risk of resistance and improve potency against Gram-positive bacteria. A new third-generation formulation, levofloxacin 1.5%, was recently introduced, demonstrating increased ocular penetration compared with gatifloxacin 0.3% but clinical equivalence to its second-generation parent, ofloxacin 0.3%, in two randomized trials.

***Methods*:** We investigated the therapeutic potential of levofloxacin 1.5% and compared it to that of existing fourth-generation fluoroquinolones. A MEDLINE search was conducted using the following search terms: moxifloxacin or gatifloxacin; levofloxacin; minimum inhibitory concentration or prevention or prophylaxis; keratitis or endophthalmitis.

***Results*:** Nine eligible studies published between 2002 and 2008 were identified, eight of which are presented. The five in-vitro studies demonstrated that moxifloxacin and gatifloxacin are statistically more potent than levofloxacin against Gram-positive organisms and similar in potency in most cases of Gram-negative bacteria. In-vivo animal models testing moxifloxacin or gatifloxacin against levofloxacin 0.5% (no clinical trials testing the effica-

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cy of levofloxacin 1.5% have yet been published) demonstrated that fourth-generation agents were superior to third-generation levofloxacin 0.5% for prophylaxis of Gram-positive bacteria-induced infections and were equal to, or better than, levofloxacin 0.5% for the treatment of Gram-negative infections.

***Conclusion*:** Fourth-generation agents have increased potency against Gram-positive bacteria compared with levofloxacin, while maintaining similar potency against Gram-negative bacteria. Although levofloxacin 1.5% has demonstrated superior ocular penetration relative to gatifloxacin, the limited available data do not suggest this translates into superior clinical activity compared with moxifloxacin, which has significantly greater ocular penetration and better Gram-positive potency than gatifloxacin.

Keywords: antibiotic susceptibility; endophthalmitis; fluoroquinolones; gatifloxacin; keratitis; levofloxacin; moxifloxacin; penetration; potency

INTRODUCTION

Since their introduction in the 1990s, the topical fluoroquinolones have gained widespread use against ocular infections, with second-generation agents ciprofloxacin 0.3% (Ciloxan®; Alcon Laboratories, Inc., Fort Worth, TX, USA) and ofloxacin 0.3% (Ocuflox®; Allergan, Inc., Irvine, CA, USA) earning US Food and Drug Administration (FDA) approval for the treatment of bacterial conjunctivitis and keratitis.[1,2] Furthermore, these agents are routinely used off-label for surgical prophylaxis.[3] These fluoroquinolones act by inhibiting topoisomerases, enzymes that are essential to bacterial DNA synthesis.[4] The functional inactivation of these enzymes ultimately results in rapid bacterial cell death.[5] In most cases, DNA gyrase (topoisomerase II) is the primary target for these fluoroquinolones in Gram-negative organisms, whereas topoisomerase IV is the main target in Gram-positive bacteria.[4] The antimicrobial spectrum of these second-generation fluoroquinolones includes Gram-positive and most Gram-negative organisms.[6] However, the in-vitro potency of these agents among bacteria isolated from both keratitis and endophthalmitis cases has been decreasing steadily due to increasing resistance since their introduction, particularly among Gram-positive organisms.[7-10]

A third-generation fluoroquinolone, levofloxacin 0.5% (Quixin®; Vistakon Pharmaceuticals, Jacksonville, FL, USA), was introduced in 2000; this agent is the pure L-enantiomer of the racemic drug ofloxacin.[11] This purified mixture is more water soluble than ofloxacin at neutral pH,[11] allowing it to be formulated at a higher concentration than ofloxacin or ciprofloxacin. This in turn produces higher ocular tissue concentrations,[12-14] creating a theoretical improvement in

clinical efficacy and an established reduction in the risk of selecting for fluoroquinolone resistance.[15] Levofloxacin also demonstrates increased activity against *Streptococci* relative to second-generation fluoroquinolones.[15-17]

Structural modifications were made in the development of the fourth-generation agents moxifloxacin (Vigamox®; Alcon Laboratories, Inc., Fort Worth, TX, USA) and gatifloxacin (Zymar®; Allergan, Inc., Irvine, CA, USA), specifically to further increase potency against Gram-positive bacteria while maintaining the broad spectrum of Gram-negative activity observed with the older fluoroquinolones.[4] Substitution of a methoxy group at position 8 of the quinolone ring accomplishes this through the simultaneous inhibition of both DNA gyrase and topoisomerase IV in Gram-positive bacteria.[18] Not only does this dual targeting of topoisomerases increase the potency of the fourth-generation agents, but it also reduces the risk of resistance because concomitant mutations in both genes are less likely to occur than the single mutation required to cause resistance to the older fluoroquinolones.[19-23] Another advantage of the 8-methoxy fluoroquinolone structure is a reduced susceptibility to efflux from the bacterial cell, further reducing the risk of resistance.[24] The efficacy produced by these structural modifications is illustrated by a recent retrospective study by Ogawa and colleagues, reporting zero cases of endophthalmitis in over 5700 cataract surgeries using moxifloxacin as prophylaxis.[25]

Recently, a new formulation of the third-generation levofloxacin has been approved by the FDA; levofloxacin 1.5% (Iquix®; Vistakon Pharmaceuticals, Jacksonville, FL, USA) is three times more concentrated than the original formulation of 0.5%. Clinically, in two randomized controlled trials of 280 culture-positive eyes reported in the package insert for levofloxacin 1.5%, this drug produced a clinical cure rate that was numerically inferior and statistically equivalent to its second-generation parent fluoroquinolone, ofloxacin 0.3% (80% vs. 84%, respectively).[26] The minimum inhibitory concentrations (MICs) of levofloxacin against specific bacterial pathogens are not impacted by drug concentration; thus, the MICs of levofloxacin 1.5% and 0.5% are identical. However, the increased concentration of levofloxacin 1.5% may improve its ability to penetrate ocular tissues. We were interested to determine whether evidence exists suggesting that this newly approved drug has any potential advantages compared with the fourth-generation fluoroquinolones.

While gatifloxacin 0.3% and levofloxacin 0.5% were shown to have nearly identical conjunctival concentrations in a study by Wagner et al,[27] the 1.5% formulation of levofloxacin had significantly greater corneal and aqueous humor concentrations than gatifloxacin in a separate study;[28] the implication, although not formally demonstrated in a head-to-head comparison, is that levofloxacin 1.5% has superior penetration compared with levofloxacin 0.5%. Since the bactericidal activity of fluoroquinolones is concentration-dependent,[29] this apparent increased intraocular penetration of levofloxacin 1.5% may lead to superior

antimicrobial activity, at least against those particular pathogens with MICs greater than the tissue concentrations achieved by the 0.5% formulation.

In order to more carefully investigate the therapeutic potential of this new third-generation agent and to compare it with that of existing fourth-generation fluoroquinolones, we conducted a MEDLINE search of studies that examined the in-vitro and/or in-vivo attributes of levofloxacin to moxifloxacin and/or gatifloxacin.

METHODS

Search and Selection Criteria

A MEDLINE search through June 2008 (with no lower time limit imposed) was conducted using the following search terms: moxifloxacin or gatifloxacin; levofloxacin; minimum inhibitory concentration or prevention or prophylaxis; keratitis or endophthalmitis. Results were limited to comparative studies. Only studies that directly compared levofloxacin to moxifloxacin and/or gatifloxacin were included, as these products are the only third- and fourth-generation fluoroquinolones used in ophthalmology.

Data Extraction

From each published study, the following data were extracted, if available: test subjects (human or animal), type of infection (keratitis or endophthalmitis), number of isolates, strain(s) of isolates, fluoroquinolones tested, assay method(s), comparative study results, and statistics.

RESULTS

Nine eligible studies published between 2002 and 2008 were identified.[30-38] Six examined the in-vitro efficacies of fluoroquinolones against human bacterial isolates,[30-35] and three presented the use of fluoroquinolones in animal models of endophthalmitis or keratitis.[36-38] The in-vitro publications reported susceptibility or resistance rates that are not presented in this review. This decision was made because of a lack of consistency in the breakpoints reported across these studies; three different breakpoint standards were used, and in the case of one study, no information on the breakpoints was provided. This variance made it impossible to compare susceptibility rates across studies; therefore we chose to present standardized MIC data, which was reported in five of the six in-vitro studies (the sixth study was excluded from analysis). Eight studies are presented in detail below.

In-Vitro Antibacterial Activity

Three of the five in-vitro studies reported MICs of different bacterial strains from endophthalmitis or keratitis isolates. In each study, the MICs of five fluoroquinolones, including levofloxacin, moxifloxacin, and gatifloxacin, were determined using E-tests (AB Biodisk, Piscataway, NJ, USA, where specified). The first of these studies measured MICs of 123 bacterial endophthalmitis and keratitis isolates.[30] As shown in Table 1, the fourth-generation fluoroquinolones had lower median MICs (range, 4.0-fold to 10.7-fold) than levo-

Table 1. Median minimum inhibitory concentrations (μg/mL) of bacterial endophthalmitis and keratitis isolates to fluoroquinolones.[30]

Bacterial isolates	*n*	Moxifloxacin	Gatifloxacin	Levofloxacin
Gram-positive ciprofloxacin-susceptible	19	0.094	0.094	0.38
Gram-positive ciprofloxacin-intermediate	17	0.38	0.5	1.5
Gram-positive ciprofloxacin-resistant	32	1.75	1.5	16
Gram-negative ciprofloxacin-susceptible	26	2	0.5	0.5
Gram-negative ciprofloxacin-intermediate	1	1.5	4	4
Gram-negative ciprofloxacin-resistant	28	32	32	32

Table 2. Median minimum inhibitory concentrations (μg/mL) of bacterial endophthalmitis isolates to fluoroquinolones.[31]

Bacterial isolates	*n*	Moxifloxacin (mox)	Gatifloxacin (gat)	Levofloxacin (lev)	Potency by rank ($P<0.05$)
Staphylococcus aureus FQR	8	1.75	3.5	12	mox>gat>lev
Staphylococcus aureus FQS	6	0.06	0.11	0.22	mox>gat>lev
Coag-neg *Staphylococcus* FQR	10	2.5	2	38	mox=gat>lev
Coag-neg *Staphylococcus* FQS	10	0.05	0.09	0.13	mox>gat>lev
Streptococcus pneumoniae	10	0.09	0.22	0.63	mox>gat>lev
Streptococcus viridans	10	0.13	0.25	0.75	mox>gat>lev
Beta-hem Streptococcus	5	0.13	0.25	0.75	mox>gat>lev
Enterococcus species	9	0.19	0.38	0.75	mox>gat>lev
Bacillus species	9	0.09	0.09	0.13	mox=gat>lev

Coag-neg=coagulase-negative; FQR=fluoroquinolone-resistant (ciprofloxacin and ofloxacin); FQS=fluoroquinolone-sensitive (ciprofloxacin and ofloxacin).

floxacin against Gram-positive bacteria and equivalent median MICs against Gram-negative isolates.

Mather et al. measured the fluoroquinolone MICs of 93 bacterial endophthalmitis isolates.[31] All Gram-positive organisms tested had significantly lower median MICs for moxifloxacin and gatifloxacin than for levofloxacin (Table 2). Furthermore, in all but one type of organism (fluoroquinolone-resistant *Staphylococcus aureus*), levofloxacin had median MICs that were not statistically different from second-generation ciprofloxacin. Finally, between the two fourth-generation agents, moxifloxacin had significantly lower median MICs for nearly all types of Gram-positive isolates (only *Bacillus* species and fluoroquinolone-resistant coagulase-negative *Staphylococcus* were similar to gatifloxacin). Gram-negative isolates were equally susceptible to moxifloxacin, gatifloxacin, and levofloxacin.

Table 3. Median minimum inhibitory concentrations (MICs; μg/mL) of bacterial keratitis isolates to fluoroquinolones.[32]

Bacterial isolates	*n*	Moxifloxacin (mox)	Gatifloxacin (gat)	Levofloxacin (lev)	Potency by rank ($P<0.05$)
Gram-positive bacteria					
Staphylococcus aureus FQR	25	1.5	4	16	mox>gat>lev
Staphylococcus aureus FQS	25	0.032	0.094	0.19	mox>gat>lev
Coag-neg *Staphylococcus* FQR	10	2.5	3	64	mox=gat>lev
Coag-neg *Staphylococcus* FQS	10	0.064	0.125	0.19	mox>gat>lev
Streptococcus pneumoniae	20	0.125	0.22	0.75	mox>gat>lev
Streptococcus viridans	20	0.125	0.25	0.75	mox>gat>lev
Gram-negative bacteria					
Pseudomonas aeruginosa FQR	12	Resistant to all fluoroquinolones			
Pseudomonas aeruginosa FQS	25	0.5	0.25	0.38	gat>lev>mox
Serratia marcescens	10	0.25	0.25	0.19	mox=gat=lev
Haemophilus species	10	0.039	0.017	0.024	gat=lev>mox
Moraxella species	10	0.047	0.032	0.047	gat>mox>lev

Note: analysis ranked all MICs from lowest to highest and compared the antibiotics by analysis of variance (ANOVA) of the ranks (not the actual MICs) using Duncan's multiple comparisons at $P<0.05$ significance. Coag-neg=coagulase-negative; FQR=fluoroquinolone-resistant (ciprofloxacin and ofloxacin); FQS=fluoroquinolone-sensitive (ciprofloxacin and ofloxacin).

Kowalski and colleagues measured the MICs of 177 bacterial keratitis isolates.[32] Results between third- and fourth-generation agents were similar to those reported by Mather et al. In all Gram-positive organisms tested, moxifloxacin and gatifloxacin had significantly lower median MICs than levofloxacin (Table 3). However, unlike in Mather et al., four of the six Gram-positive strains showed lower levofloxacin median MICs than those of the second-generation agents. Results from Gram-negative organisms were roughly similar among gatifloxacin, moxifloxacin, and levofloxacin, although one or both fourth-generation fluoroquinolones had significantly lower median MICs than levofloxacin for *Moraxella* species and fluoroquinolone-susceptible *Pseudomonas aeruginosa*.

The remaining two in-vitro articles investigated the antimicrobial activity of fluoroquinolones against a singular bacterial strain. The first of these manuscripts reported the in-vitro efficacies of fluoroquinolones against coagulase-negative *Staphylococcus* isolates,[33] the most frequent type of bacterial pathogen responsible for postoperative endophthalmitis.[39] Both E-tests (AB Biodisk, Piscataway, NJ, USA) and disk diffusion assays (antibiotic-impregnated paper disks; Becton Dickinson,

Cockeysville, MD, USA) were performed to determine the MICs of these isolates. For the time period 2000-2004, moxifloxacin (0.12 μg/mL) and gatifloxacin (0.19 μg/mL) had the lowest median MICs, followed by levofloxacin, ciprofloxacin, and ofloxacin, all of which had median MICs of 0.50 μg/mL.

Finally, the in-vitro susceptibility of 12 bacterial keratitis isolates of ciprofloxacin-resistant *Pseudomonas aeruginosa* were tested against a number of antibiotics, including fluoroquinolones.[34] In keeping with the results from the larger study from Kowalski and colleagues,[32] all 12 isolates were resistant to all five fluoroquinolones (median MICs >32 μg/mL).

In-Vivo Antibacterial Activity

Using a placebo-controlled rabbit model of fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus*-induced endophthalmitis, Kowalski and colleagues tested the prophylactic abilities of moxifloxacin 0.5% and levofloxacin 0.5%.[36] Rabbits were treated with one drop of study medication every 15 minutes for 1 hour prior to bacterial challenge (five drops total). At 24 hours post-inoculation, the presence or absence of endophthalmitis was judged both by slit-lamp examination and by culturing samples from aqueous humor and vitreous. Clinical examination revealed that moxifloxacin

Figure 1. Prophylactic activity of moxifloxacin 0.5% and levofloxacin 0.5% in a rabbit model of fluoroquinolone-resistant, methicillin-resistant *Staphylococcus aureus*-induced endophthalmitis.[36]

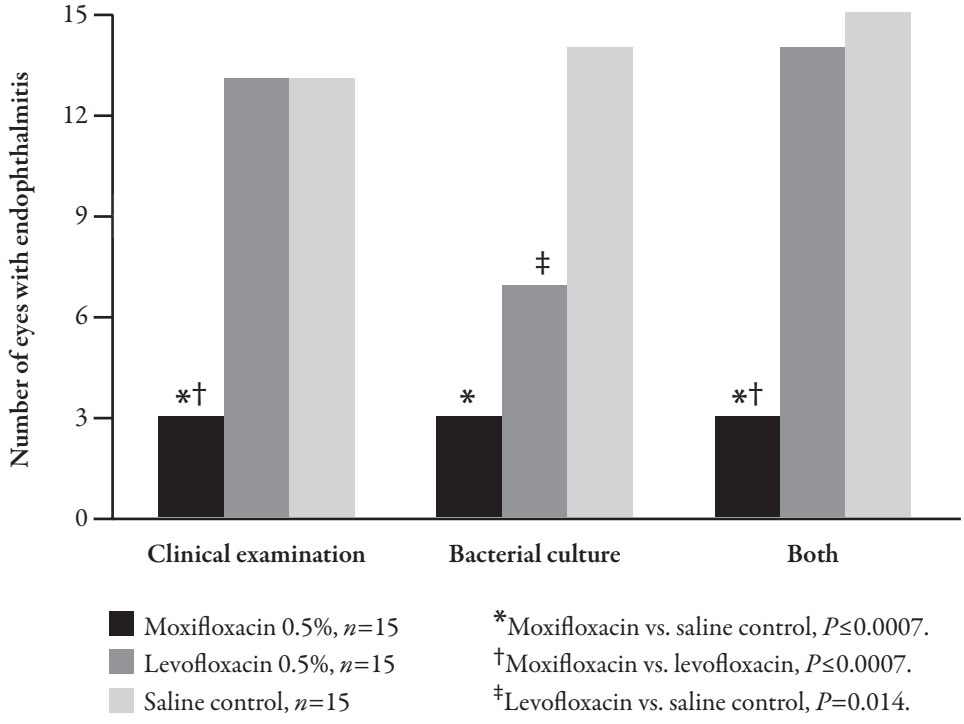

Table 4. Efficacy outcomes in a rabbit model of bacterial keratitis.[38]

Efficacy score	Gatifloxacin 0.3% (*n*=7)	Levofloxacin 0.5% (*n*=7)	BSS control (*n*=7)
Keratitis-positive eyes	0	5	5
Mean ocular inflammation scores	4.86±2.72*	12.64±7.20	17.21±6.66

*P=0.020, gatifloxacin vs. levofloxacin; P<0.001, gatifloxacin vs. balanced salt solution (BSS) control.

prevented more infections (12/15) than levofloxacin (2/15, P=0.0007; Figure 1). Moreover, slit-lamp examination results from levofloxacin-treated eyes were virtually indistinguishable from that of the saline controls, in both the number of eyes with endophthalmitis (13/15 in each group) and in clinical symptom scores such as conjunctivitis, iritis, and cells and flare. When clinical examination results were combined with results from the bacterial cultures, moxifloxacin still prevented endophthalmitis in 12 of 15 eyes, but levofloxacin prophylaxis was successful in only one eye (P=0.0001).

The second in-vivo study examined the effectiveness of fluoroquinolones against *Pseudomonas aeruginosa* and *Serratia marcescens* in a rabbit model of keratitis.[37] Rabbits were divided into five treatment groups for each strain: moxifloxacin 0.5%, levofloxacin 0.5%, ciprofloxacin 0.3%, ofloxacin 0.3%, and untreated control. From 16 to 22 hours post-inoculation, rabbits were treated with one drop of study drug every 30 minutes. At 23 hours post-inoculation, corneas were harvested and cultured. For *Serratia marcescens* infections, moxifloxacin-treated eyes had a significantly greater reduction in colony-forming units (CFUs) from untreated controls than levofloxacin-treated eyes. Moxifloxacin and levofloxacin had equivalent activity against *Pseudomonas aeruginosa.* This study also reported on the fluoroquinolone median MICs of each strain: *Pseudomonas aeruginosa* was more susceptible to levofloxacin than moxifloxacin (0.5 μg/mL vs. 1.04 μg/mL), while *Serratia marcescens* was equally susceptible to both fluoroquinolones (0.25 μg/mL vs. 0.27 μg/mL, respectively).

Tungsiripat et al. used a placebo-controlled rabbit model of *Staphylococcal*-induced keratitis to compare the prophylactic activities of gatifloxacin 0.3%, levofloxacin 0.5%, and ciprofloxacin 0.3%.[38] Following intraocular inoculation with 1000 CFUs of multiple drug-resistant *Staphylococcus aureus*, rabbits were topically treated with study medication immediately, 6, 12, and 18 hours after surgery. Clinically apparent infection was determined using slit-lamp examination. Six inflammatory factors—conjunctival injection, conjunctival chemosis, iritis, hypopyon, and stromal edema—were also judged using slit-lamp examination; scores were combined to get an overall inflammation score for each eye. Gatifloxacin completely prevented clinical infection (n=7), whereas levofloxacin was no better than balanced salt solution at prophylaxis, with both groups having five of seven eyes

develop keratitis (Table 4). Moreover, at 24 hours post-inoculation, the mean inflammation score for gatifloxacin-treated eyes (4.86±2.72) was significantly lower than that of levofloxacin-treated eyes (12.64±7.20; P=0.02) and control eyes (17.21±6.66; P<0.001).

DISCUSSION

The studies reviewed here demonstrate that the in-vitro Gram-positive actions of fourth-generation fluoroquinolones (gatifloxacin, moxifloxacin) are superior to those of third-generation levofloxacin. The median MICs of both fourth-generation agents were consistently lower than those of levofloxacin for each Gram-positive pathogen reported, and moxifloxacin was significantly more potent than gatifloxacin in the majority of these strains.[30-32] Of note, in many cases, levofloxacin median MICs were statistically indistinguishable from those of ciprofloxacin, suggesting that levofloxacin is not more potent against Gram-positive pathogens than a second-generation agent.[31,32] These results would be applicable to both formulations of levofloxacin (0.5% and 1.5%), as potency does not vary with changes in drug concentration. Thus, levofloxacin 1.5% appears to have no potency advantage over fourth-generation fluoroquinolones.

The increased potency against Gram-positive bacteria of the fourth-generation fluoroquinolones is clinically relevant because Gram-positive pathogens are the most prevalent organisms identified in both endophthalmitis and keratitis isolates.[39-41] *Staphylococcus aureus* and coagulase-negative *Staphylococcus* are the most frequently identified Gram-positive pathogens for both types of infection.[39,42] Based on the results presented in this review, the fourth-generation fluoroquinolones are 1.4-fold to 19-fold more potent than levofloxacin against these particular organisms.[31-33]

Gram-negative pathogens are responsible for only a minority of all endophthalmitis and keratitis cases.[9,39,40] Based on our review, neither third- nor fourth-generation agents emerged as superior over one another.

Aside from its potency, the drug's in-vivo activity is also highly dependent on its ability to penetrate ocular tissues. In a study comparing conjunctival concentrations of five topical fluoroquinolones, Wagner et al. reported that the concentration of moxifloxacin was significantly greater than the concentrations of the other four fluoroquinolones (P<0.001), including gatifloxacin 0.3% and levofloxacin 0.5%.[27] Two additional clinical trials report that moxifloxacin had significantly higher corneal tissue and/or aqueous humor concentrations than gatifloxacin.[43,44] Both studies arrived at the same conclusions, but the more widely accepted data for fourth-generation fluoroquinolones aqueous humor concentrations are those from Kim and colleagues,[43] owing to an important difference in the two study designs. Holland et al. used only two drops of fluoroquinolone prior to surgery, whereas Kim and colleagues employed pulse dosing, in which patients received drops 10 minutes apart for a total of four doses with the last dose given within 2 minutes of cataract

incision. Although 1-3 days of preoperative dosing is currently the most standardized approach to prophylaxis,[45] pulse dosing on the day of surgery is also a common practice.

To date, no studies examining pulsed doses of levofloxacin 1.5% prior to cataract surgery have been published. In the only study reporting on the penetration of levofloxacin 1.5%, Holland et al. again used two drops of fluoroquinolone prior to surgery, demonstrating that levofloxacin 1.5% had significantly higher corneal tissue and aqueous humor concentrations than gatifloxacin 0.3% ($P<0.0001$ and $P=0.0002$, respectively).[28] Although the conjunctival study above[27] reports the superior penetration of moxifloxacin compared with levofloxacin 0.5%, no studies have been published comparing moxifloxacin's penetration to that of levofloxacin 1.5%. However, the two penetration studies performed by Holland and colleagues (moxifloxacin 0.5% vs. gatifloxacin 0.3% and levofloxacin 1.5% vs. gatifloxacin 0.3%)[28,44] had similar study designs and thus, some insight may be gained by examining the moxifloxacin and levofloxacin arms more carefully. Both studies delivered two drops of study medication 5 minutes apart prior to aqueous humor and corneal tissue sample collection. In the moxifloxacin study, investigators collected samples at 15, 30, 60, and 120 minutes ($n=25$, total patients treated with moxifloxacin) after the final dose, whereas investigators in the levofloxacin study collected samples at 10 minutes after the final dose ($n=27$, total patients treated with levofloxacin). In corneal tissue, levofloxacin mean concentration was 64.758 μg/g (Table 5). In the moxifloxacin study, corneal tissue was dissected into epithelium, stroma, and endothelium; the respective concentrations of moxifloxacin 15 minutes after instillation were 81.2, 48.5, and 76.1 μg/g. Because moxifloxacin concentrations were measured 5 minutes later than levofloxacin (15 minutes vs. 10 minutes after instillation), moxifloxacin would be expected to have the higher concentrations at 10 min-

Table 5. Corneal and aqueous penetration data for moxifloxacin 0.5% and levofloxacin 1.5% from separate clinical studies.[28,44]

	Moxifloxacin 0.5% (Holland et al. 2008),[44] μg/g or μg/mL				Levofloxacin 1.5% (Holland et al. 2007),[28] μg/g or μg/mL
Time dosed prior to surgery:	**15 min**	**30 min**	**1 h**	**2 h**	**10 min**
Corneal epithelium	81.2	40	35	15	NA
Corneal stroma	48.5	20	15	10	NA
Corneal endothelium	76.1	35	10	10	NA
Corneal tissue	NA	NA	NA	NA	64.758
Aqueous humor	0.3	0.25	0.9	0.8	0.976

Note: Holland et al. 2008[44] concentrations at 30 minutes, 1 hour, and 2 hours are approximate.
NA=no data present.

utes. In the aqueous humor, levofloxacin 1.5% was present at 0.976 μg/mL after 10 minutes, whereas moxifloxacin reached approximately the same level at 1 hour.

The penetration data from these two studies can be combined with potency data to get an approximation of the relative expected in-vivo efficacies of moxifloxacin 0.5% and levofloxacin 1.5%. Table 6 shows the penetration:MIC ratios using MIC_{50} values (Mather et al. 2002,[31] and Kowalski et al. 2003[32]) and MIC_{90} values (Kowalski et al. 2003[32]). As shown by the numbers in bold, the moxifloxacin ratios are higher than the levofloxacin ratios for all Gram-positive pathogens tested regardless of whether corneal or aqueous penetration values were used, with only the exception of aqueous penetration:MIC ratios against one pathogen: fluoroquinolone-sensitive coagulase-negative *Staphylococcus*. This suggests empiric treatment with moxifloxacin, compared with levofloxacin, is more effective as prophylactic antibiotic.

While direct comparisons of the above data cannot be made in order to draw conclusions regarding the relative penetration or in-vivo efficacy of levofloxacin 1.5% and moxifloxacin 0.5%, they do at least provide some information suggesting that these two agents appear to have similar ocular penetration. However, given the superiority of moxifloxacin potency over levofloxacin and the clinical equivalence of levofloxacin 1.5% to second-generation ofloxacin 0.3%, these studies do not provide any compelling evidence indicating that the more concentrated form of levofloxacin should have any clinical advantage over moxifloxacin.

The contribution of penetration to the in-vivo activity of a fluoroquinolone is suggested by the results of the Thibodeaux study presented here,[37] which used a rabbit model of keratitis to investigate the effectiveness of fluoroquinolones against two Gram-negative organisms, *Pseudomonas aeruginosa* and *Serratia marcescens*. Moxifloxacin treatment produced a significantly greater reduction in CFUs cultured from *Serratia marcescens*-infected eyes compared with levofloxacin 0.5%, despite the fact that MIC results showed this strain was equally susceptible to both fluoroquinolones. Similarly, although the *Pseudomonas aeruginosa* strain was shown to be more susceptible to levofloxacin than moxifloxacin, the two fluoroquinolones produced equivalent reductions in CFUs from untreated controls in eyes infected with this pathogen. These differences between in-vitro and in-vivo activity may be explained by the ability of moxifloxacin to penetrate ocular tissues, and suggests that penetration plays a role in the clinical efficacy of topical fluoroquinolones.

As previously discussed, the package insert for this new fluoroquinolone reports the results of 280 culture-positive eyes treated with either levofloxacin 1.5% or second-generation ofloxacin 0.3%, in which levofloxacin 1.5% produced a clinical cure rate that was no better than ofloxacin 0.3% (80% vs. 84%, respectively).[26] Since the potency of this new formulation remains the same as levofloxacin 0.5%, any increase in clinical activity would be expected to arise from improved penetration. However, these results showing no clinical improvement over its second-

Table 6. Penetration:minimum inhibitory concentrations (MIC) ratio data using Gram-positive pathogens for moxifloxacin 0.5% and levofloxacin 1.5% from separate clinical studies.[28,31,32,44]

	Using Mather et al.[31] MIC_{50}				Using Kowalski et al.[32] MIC_{50}				Using Kowalski et al.[32] MIC_{90}			
	Cornea		Aqueous humor		Cornea		Aqueous humor		Cornea		Aqueous humor	
Gram-positive pathogen	**mox**	**lev**	**mox**	**lev**	**mox**	**lev**	**mox**	**lev**	**mox**	**lev**	**mox**	**lev**
Staphylococcus aureus FQR	**39.20**	5.40	**0.17**	0.08	**45.73**	4.05	**0.20**	0.06	**17.15**	2.02	**0.08**	0.03
Staphylococcus aureus FQS	**1143.33**	294.35	**5.00**	4.44	**2143.75**	340.83	**9.38**	5.14	**1459.57**	170.42	**6.38**	2.37
Coag-neg *Staphylococcus* FQR	**27.44**	1.70	**0.12**	0.03	**27.44**	1.01	**0.12**	0.02	**22.87**	1.01	**0.10**	0.01
Coag-neg *Staphylococcus* FQS	**1372.00**	498.14	6.00	**7.51**	**1071.88**	340.83	4.69	**5.14**	**548.80**	340.83	2.40	**4.74**
Streptococcus pneumoniae	762.22	102.79	**3.33**	1.55	**548.80**	86.34	**2.40**	1.30	**361.05**	64.76	**1.58**	0.90
Streptococcus viridans	**527.69**	86.34	**2.31**	1.30	**548.80**	86.34	**2.40**	1.30	**361.05**	64.76	**1.58**	0.90

Penetration data for moxifloxacin from Holland et al. 2008[44] using the average of all three corneal tissues at 15-minute time points (68.6 μg/g).
Penetration data for levofloxacin 1.5% from Holland et al. 2007.[28]
MIC data from Mather et al. 2002[31] and Kowalski et al. 2003.[32]
Coag-neg=coagulase-negative; FQR=fluoroquinolone-resistant (ciprofloxacin and ofloxacin); FQS=fluoroquinolone-sensitive (ciprofloxacin and ofloxacin); lev=levofloxacin; mox=moxifloxacin.

generation parent fluoroquinolone suggest that perhaps one drop of levofloxacin 1.5% is functionally equivalent to three drops of levofloxacin 0.5%.

Although the relative clinical efficacy in humans of levofloxacin 1.5% compared to either fourth-generation fluoroquinolone is yet unknown, the animal studies described in this paper reporting on prophylaxis by fluoroquinolones demonstrate that the fourth-generation agents have superior in-vivo antimicrobial activity compared with levofloxacin 0.5%.[36,38] Two animal studies examining the abilities of fluoroquinolones to treat keratitis also illustrate the superiority of fourth-generation agents.[46,47]

While efficacy is important, safety must also be considered. The most recent, peer-reviewed publication tested fluoroquinolone toxicity in corneal and conjunctival cells.[48] Results showed that moxifloxacin had less corneal and conjunctival toxicity than all other fluoroquinolones tested, including gatifloxacin 0.3% and levofloxacin 0.5%, which were not significantly different from one another.

The clinical significance of this analysis is limited by the paucity of in-vivo data comparing all third- and fourth-generation fluoroquinolones within a controlled setting. Aside from the one clinical penetration study evaluating gatifloxacin and levofloxacin 1.5% by Holland and colleagues,[28] no comparative studies using levofloxacin 1.5% have been published to date. Only three in-vivo studies were identified in the current MEDLINE search, all of which used animal models. Thus, the relative clinical utilities of third- and fourth-generation fluoroquinolones must currently be assessed from available potency and penetration studies. Additional clinical trials comparing moxifloxacin, gatifloxacin, and levofloxacin 0.5% and 1.5% are needed to draw definitive conclusions regarding the relative clinical efficacies of these fluoroquinolones.

CONCLUSION

In summary, fourth-generation agents show statistically better Gram-positive potency compared with levofloxacin while maintaining similar Gram-negative potency. Although levofloxacin 1.5% has demonstrated superior ocular penetration relative to gatifloxacin, available data do not suggest this translates into superior clinical activity compared with moxifloxacin, as moxifloxacin has been shown to have significantly greater penetration into the ocular tissues and better Gram-positive coverage compared with gatifloxacin. Head-to-head in-vivo comparisons of levofloxacin 1.5%, gatifloxacin, and moxifloxacin are needed to confirm this hypothesis.

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