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REVIEW

Besifloxacin Ophthalmic Suspension, 0.6%: a Novel Topical Fluoroquinolone for Bacterial Conjunctivitis

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ABSTRACT

Acute bacterial conjunctivitis, the most common cause of conjunctivitis, is responsible for approximately 1% of all primary-care consultations. Of the topical ophthalmic antibiotics used to treat acute bacterial conjunctivitis, fluoroquinolones are especially useful because they possess a broad antibacterial spectrum, are bactericidal in action, are generally well tolerated, and have been less prone to development of bacterial resistance. Besifloxacin, the latest advanced fluoroquinolone approved for treating bacterial conjunctivitis, is the first fluoroquinolone developed specifically for topical ophthalmic use. It has a C-8 chlorine substituent and is known as a chloro-fluoroquinolone. Besifloxacin possesses relatively balanced dual-targeting activity against bacterial topoisomerase IV and DNA gyrase (topoisomerse II), two essential enzymes involved in bacterial DNA replication, leading to increased potency and decreased likelihood of bacterial resistance developing to besifloxacin. Microbiological data suggest a relatively high potency and rapid bactericidal activity for besifloxacin against common ocular pathogens, including bacteria resistant to other fluoroquinolones, especially resistant staphylococcal species. Randomized, double-masked, controlled clinical studies demonstrated the clinical efficacy of besifloxacin ophthalmic suspension 0.6% administered three-times daily for 5 days to be superior to the vehicle alone and similar to moxifloxacin ophthalmic solution 0.5% for bacterial conjunctivitis. In addition, besifloxacin ophthalmic suspension 0.6% administered two-times daily for 3 days was clinically more effective than the vehicle alone for bacterial conjunctivitis. Besifloxacin has also been shown in preclinical animal studies to be potentially effective for the "off-label" treatment of infections following ocular surgery, prophylaxis of endophthalmitis, and the treatment of bacterial keratitis. Taken together,

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clinical and preclinical animal studies indicate that besifloxacin is an important new option for the treatment of ocular infections.

Keywords: Acute bacterial conjunctivitis; Bacterial resistance; Besifloxacin; Endophthalmitis; Fluoroquinolones; Keratitis

INTRODUCTION

Acute bacterial conjunctivitis is an inflammation of the mucous membrane that covers the white of the eye and inner eyelids and is characterized by purulent to mucopurulent discharge, redness, and a gritty feeling. It is a common ocular surface infection. A study of patient visits to community health centers in the United Kingdom concluded that approximately 1% of all primary-care consultations are for acute bacterial conjunctivitis [1]. The organism most often associated with acute bacterial conjunctivitis in adults is *Staphylococcus aureus*. *Haemophilus influenzae*, *Streptococcus pneumoniae,* and *Moraxella* species are other commonly isolated pathogens [2]. In children, *H. influenzae*, *S. pneumoniae, S. aureus,* and *Moraxella catarrhalis* are the most common causative pathogens [2].

Although most episodes of acute bacterial conjunctivitis resolve clinically in about 7 days without therapy [3], empirical treatment with topical ophthalmic antibiotics provides a number of benefits for patients [4]. Clinical and microbiological remission rates are significantly improved and more patients experience remission faster, usually within 2–5 days, with empirical treatment [4]. Thus, topical antibiotic treatment can diminish time lost from school or work and minimize employee or student furloughs. Other benefits of early empirical topical antibiotic therapy include lowering the risk of transmission of this contagious infection and decreasing the potential for sight-threatening complications, such as orbital cellulitis, panophthalmitis, and bacterial keratitis. For people who wear contact lenses, treatment can decrease the time during which they cannot wear their contact lenses [4–6]. The classes of antibiotics used to treat acute bacterial conjunctivitis include the sulfonamide antibiotics (sulfacetamide), aminoglycosides (gentamicin and tobramycin), polymyxin B-based formulations (bacitracin and polymyxin B in combinations with trimethoprim, neomycin, sulfacetamide, or gramicidin), macrolides (erythromycin and azithromycin), and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, moxifloxacin, and besifloxacin) [7–9].

Fluoroquinolones are broad-spectrum antibiotics with activity against both Gram-positive and Gram-negative pathogens associated with bacterial conjunctivitis [10, 11]. The antibacterial activity of fluoroquinolones is based primarily on targeting two enzymes, bacterial DNA gyrase (topoisomerase II) and bacterial topoisomerase IV, involved in the regulation of DNA metabolism. DNA gyrase has many actions, including catalysis of negative supercoiling of DNA, while DNA topoisomerase IV has been shown to be involved in decatenation [12]. Fluoroquinolones act to prevent bacterial DNA replication by inhibiting bacterial DNA gyrase and topoisomerase IV in susceptible organisms, [12] thereby resulting in lethally entangled DNA. Older fluoroquinolones such as ciprofloxacin and ofloxacin bind preferentially to one of the two bacterial enzymes [13]. In contrast, the newer fluoroquinolones, moxifloxacin, gatifloxacin, and besifloxacin, have structural modifications that act to confer more balanced binding of bacterial DNA gyrase and topoisomerase IV. The balanced binding increases activity of the newer fluoroquinolones against the Gram-positive pathogens associated with bacterial conjunctivitis, while retaining activity against Gram-negative pathogens [10–12, 14, 15].

METHODS

A search of the English-language literature was conducted using PubMed, employing "besifloxacin" as the single search term. There were no time limitations placed on the search window, and papers were selected if they included relevant microbiologic, pharmacokinetic, preclinical, or clinical data regarding besifloxacin's antibacterial activity, efficacy, or safety.

BACTERIAL RESISTANCE

To varying degrees, all classes of antibiotics used in the treatment of bacterial conjunctivitis have been affected by increasing rates of bacterial resistance [16]. During the past 20 years, surveillance studies of bacterial resistance among ocular infections such as The Surveillance Network (TSN), the ocular Tracking Resistance in U.S. Today (TRUST), the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR), and numerous regional studies have documented increasingly higher rates of bacterial resistance [17–20]. As an example, a surveillance study conducted in Brazil did not identify any resistance to tobramycin and gentamicin among *S. pneumoniae* cultures obtained from the cornea or conjunctiva between 1989 and 1992. In contrast, between 1997 and 2000, 56.4% and 54% of corneal and conjunctival *S. pneumoniae* cultures, respectively, were resistant to tobramycin, and 57.7% and 44%, respectively, were resistant to gentamicin [21]. A surveillance study based on 12,134 bacterial conjunctivitis cultures obtained in New York City from January 1997 through June 2008 found statistically significant annual increases in bacterial resistance to the combination antibiotic trimethoprim/sulfamethoxazole. Resistance by Gram-positive bacteria increased from about 5% to approximately 25%, and resistance among Gram-negative bacteria rose from about 15% to slightly more than 50% during the 11.5-year study period [22]. Data from a 10-year study in South Florida showed that resistance among 567 bacterial conjunctivitis isolates to the macrolide antibiotic erythromycin increased from 22.1% in January 1994 to 45.1% by December 2003 [19].

Until recent years, little resistance to fluoroquinolones was encountered among ocular pathogens, and as a result, this class emerged as a frontline treatment for bacterial conjunctivitis. Unfortunately, even resistance patterns to fluoroquinolones are changing. Multidrug-resistant strains of methicillin-resistant *S. aureus* (MRSA) have emerged that are resistant to most fluoroquinolones, especially older ones, such as ciprofloxacin, ofloxacin, and levofloxacin [18, 23]. Of MRSA isolates tested in TSN studies between 2000 and 2005, only 27–32% were susceptible to ciprofloxacin, gatifloxacin, levofloxacin, and moxifloxacin [18]. More than 65% of 111 ciprofloxacin-resistant coagulase-negative *Staphylococcus* isolates recovered from 1990 to 2004 were reported to be resistant to gatifloxacin and moxifloxacin [23].

These data highlight the need for the continuing development of new fluoroquinolones that incorporate structural features and usage characteristics that potentially minimize bacterial resistance.

BESIFLOXACIN

Besifloxacin was approved in the United States and Canada in 2009 for treating bacterial conjunctivitis and is the first fluoroquinolone developed specifically for topical ophthalmic use without having been approved previously

for a systemic indication [16]. It has also never been used outside of medicine in agriculture or animal husbandry, according to the manufacturer (Bausch & Lomb, oral communication, May 2012). Besifloxacin is a novel 8-chloro-fluoroquinolone, whose structure is shown in Fig. 1. Similar to gatifloxacin and moxifloxacin, the besifloxacin molecule contains an N-cyclopropyl group that confers broad-spectrum antimicrobial activity [24]. However, besifloxacin is the only fluoroquinolone with a C-8 chlorine substituent, which, in theory, decreases the risk of bacterial resistance development [24, 25], while increasing the drug's potency through a strong, balanced affinity for the bacterial DNA gyrase and topoisomerase IV enzymes [24]. Furthermore, the bulky amino-azepinyl substituent on C-7 may also contribute to targeting of DNA gyrase [12], as well as to besifloxacin's greater broad-spectrum activity and higher potency against Gram-positive organisms [24].

The formulation of besifloxacin marketed in the United States and Canada and recently launched in Latin America (Besivance™, besifloxacin ophthalmic suspension 0.6%, Bausch & Lomb, Inc., Rochester, NY, USA) also includes a mucoadhesive polymer delivery system (DuraSite®, InSite Vision Inc., Alameda, CA, USA) that prolongs the residence time of the drug on the ocular surface [26], an action that may increase ocular surface concentrations and enhance antibacterial efficacy through concentration-dependent killing mechanisms. Moreover, the preservative included in the formulation, 0.01% benzalkonium chloride (BAK), possesses both bacteriostatic and bactericidal activities [27, 28], although the clinical relevance of BAK has yet to be determined due to rapid dilution once instilled into the tear film [16, 29, 30].

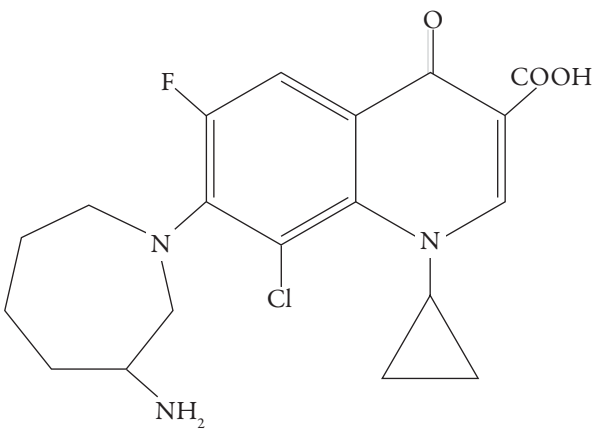


Fig. 1 Chemical structure of besifloxacin (7-[(3R)-3-aminohexahydro-1H-azepin-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid)

Microbiology

As mentioned above, the antibacterial activity of fluoroquinolones results from the inhibition of one or both of the essential bacterial replication enzymes, DNA gyrase and topoisomerase IV. In contrast to older fluoroquinolones, besifloxacin's primary mechanism of action has been shown to involve potent, balanced dual-targeting activity against both enzymes [12]. The concentration required to inhibit 50% of isolates (IC_{50}) of besifloxacin against *S. pneumoniae* DNA gyrase was 16-fold lower than that of ciprofloxacin and fourfold lower than that of moxifloxacin. Against topoisomerase IV, the besifloxacin IC_{50} was fivefold and 2.5-fold lower than ciprofloxacin and moxifloxacin, respectively. In addition to its improved potency, besifloxacin had similar activity against DNA gyrase and topoisomerase IV with the ratio of the IC_{50}s approaching 1, suggesting a balanced activity of besifloxacin against these essential bacterial enzymes [12]. This balanced dual-targeting activity theoretically reduces the development of bacterial resistance, because simultaneous mutations of both enzymes are considered highly unlikely. In support of this premise,

Table 1 In-vitro activity of besifloxacin and comparator anti-infectives against common ocular pathogens from three besifloxacin clinical trials [31]

	Minimum inhibitory concentration (μg/mL)						
Organism	**BES**	**CIP**	**GAT**	**LVX**	**MXF**	**OFX**	**AZM**
Staphylococcus aureus, all (n = 190)							
MIC_{50}	0.03	0.50	0.13	0.25	0.06	0.50	2.00
MIC_{90}	0.50	>8.00	4.00	8.00	2.00	>8.00	>8.00
MSSA-CR (n = 17)							
MIC_{50}	0.50	>8.00	4.00	8.00	2.00	>8.00	>8.00
MIC_{90}	2.00	>8.00	>8.00	<8.00	8.00	>8.00	>8.00
MRSA-CS (n = 9)							
MIC_{50}	0.06	0.50	0.13	0.25	0.06	0.50	>8.00
MRSA-CR (n = 17)							
MIC_{50}	0.50	>8.00	4.00	8.00	4.00	>8.00	>8.00
MIC_{90}	4.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00
Streptococcus epidermidis, all (n = 111)							
MIC_{50}	0.06	0.25	0.13	0.25	0.13	0.50	1.00
MIC_{90}	0.50	>8.00	2.00	8.00	4.00	>8.00	>8.00
MSSE-CR (n = 10)							
MIC_{50}	0.50	>8.00	2.00	8.00	4.00	>8.00	1.00
MIC_{90}	1.00	>8.00	8.00	>8.00	8.00	>8.00	>8.00
MRSE-CS (n = 27)							
MIC_{50}	0.06	0.25	0.13	0.25	0.13	0.50	>8.00
MIC_{90}	0.06	0.25	0.25	0.25	0.13	0.50	>8.00
MRSE-CR (n = 24)							
MIC_{50}	0.50	8.00	2.00	8.00	2.00	8.00	>8.00
MIC_{90}	4.00	>8.00	>8.00	>8.00	>8.00	>8.00	>8.00
Streptococcus pneumoniae, all (n = 302)							
MIC_{50}	0.06	0.50	0.25	0.50	0.13	1.00	0.13
MIC_{90}	0.125	1.00	0.50	1.00	0.125	2.00	>8.00
Haemophilus influenzae, all (n = 344)							
MIC_{50}	0.03	0.02	0.02	0.03	0.03	0.03	2.00
MIC_{90}	0.06	0.02	0.03	0.03	0.06	0.06	4.00

AZM azithromycin, *BES* besifloxacin, *CIP* ciprofloxacin, *GAT* gatifloxacin, *LVX* levofloxacin, *MIC* minimum inhibitory concentration, *MSSA-CR* methicillin-sensitive, ciprofloxacin-resistant *S. aureus*, *MRSA-CS* methicillin-resistant, ciprofloxacin-sensitive *S. aureus*, *MRSA-CR* methicillin-resistant, ciprofloxacin-resistant *S. aureus*, *MSSE-CR* methicillin-sensitive, ciprofloxacin-resistant *S. epidermidis*, *MRSE-CS* methicillin-resistant, ciprofloxacin-sensitive *S. epidermidis*, *MRSE-CR* methicillin-resistant, ciprofloxacin-resistant *S. epidermidis*, *MXF* moxifloxacin, *OFX*, ofloxacin

mutations resulting in resistance of *S. aureus* and *S. pneumoniae* to besifloxacin occurred almost two orders of magnitude less often than mutations resulting in resistance to ciprofloxacin, which preferentially binds to DNA gyrase [12].

Besifloxacin has been shown to have a broad spectrum of activity, including activity against Gram-positive, Gram-negative, and anaerobic pathogens responsible for ocular infections [31]. The in-vitro activity of besifloxacin generally exceeds or equals that of other topical antibiotics (e.g., moxifloxacin, gatifloxacin, ciprofloxacin, levofloxacin, azithromycin, and tobramycin) used for the treatment of ocular infections, including against pathogens resistant to other antibiotics, as shown in Table 1 [31].

Susceptibility patterns with besifloxacin have been extensively evaluated using three large pools of bacterial isolates [20, 31, 32]. The first pool consisted of 2,690 clinical bacterial isolates (40 species), most of which were ocular or respiratory specimens, obtained from a global central laboratory (Eurofins Medinet, Chantilly, VA, USA) and collected in the United States between 2005 and 2008 [32]. Compared with other fluoroquinolones and other classes of antibacterial agents, besifloxacin was shown to have the greatest potency against Gram-positive pathogens and anaerobes and generally equivalent activity against most Gram-negative isolates [32].

The second large pool of susceptibility data was generated during the prospective ARMOR surveillance project [20]. This surveillance study compared the activity of besifloxacin with ciprofloxacin and moxifloxacin as well as other antibiotics (e.g., azithromycin, clindamycin, oxacillin, tobramycin, and vancomycin) against Gram-positive and Gram-negative ocular clinical isolates collected in 2009 from 34 healthcare centers (i.e., three ocular centers, 22 community hospitals, and nine university hospitals) located throughout the United States. Comparisons of minimum inhibitory concentration $(MIC)_{50}$ and MIC_{90} values showed that besifloxacin was the most potent of the comparators against resistant and susceptible strains of *S. aureus*, coagulase-negative staphylococci, and *S. pneumoniae*. The activity of besifloxacin was especially notable against methicillin/oxacillin-resistant strains, which accounted for 39.0% and 52.8%, respectively, of *S. aureus* and coagulase-negative staphylococci isolates obtained during the ARMOR study. The MIC_{50} and MIC_{90}values for besifloxacin against MRSA (0.5 and 4, respectively) were comparable to those observed with vancomycin (0.5 and 1) [20].

Most recently, Haas et al. [31] reported an integrated analysis of susceptibility data based upon 1,324 bacterial conjunctivitis isolates gathered during the conduct of three besifloxacin clinical trials in the United States and Asia [31]. Susceptibility findings for the most commonly identified pathogens are presented in Table 1 [31]. Overall, besifloxacin was more potent than comparator agents, especially against Gram-positive organisms. Ciprofloxacin-resistant (CR) strains of *S. aureus* and *S. epidermidis* that showed an initial MIC >8 were put through an expanded range retest at higher drug concentrations. Compared with other tested fluoroquinolones, besifloxacin had four- to 128-fold greater potency against methicillin-sensitive *S. aureus* (MSSA)-CR, 16- to 128-fold greater activity against MRSA-CR, and eight- to 64-fold greater activity against methicillin-resistant *S. epidermidis* (MRSE)-CR.

A study on the bactericidal activity of besifloxacin against staphylococci, *S. pneumoniae*, and *H. influenzae* showed that besifloxacin had rapid bactericidal activity against these pathogens, including isolates that showed in-vitro resistance to other fluoroquinolones, β-lactams,

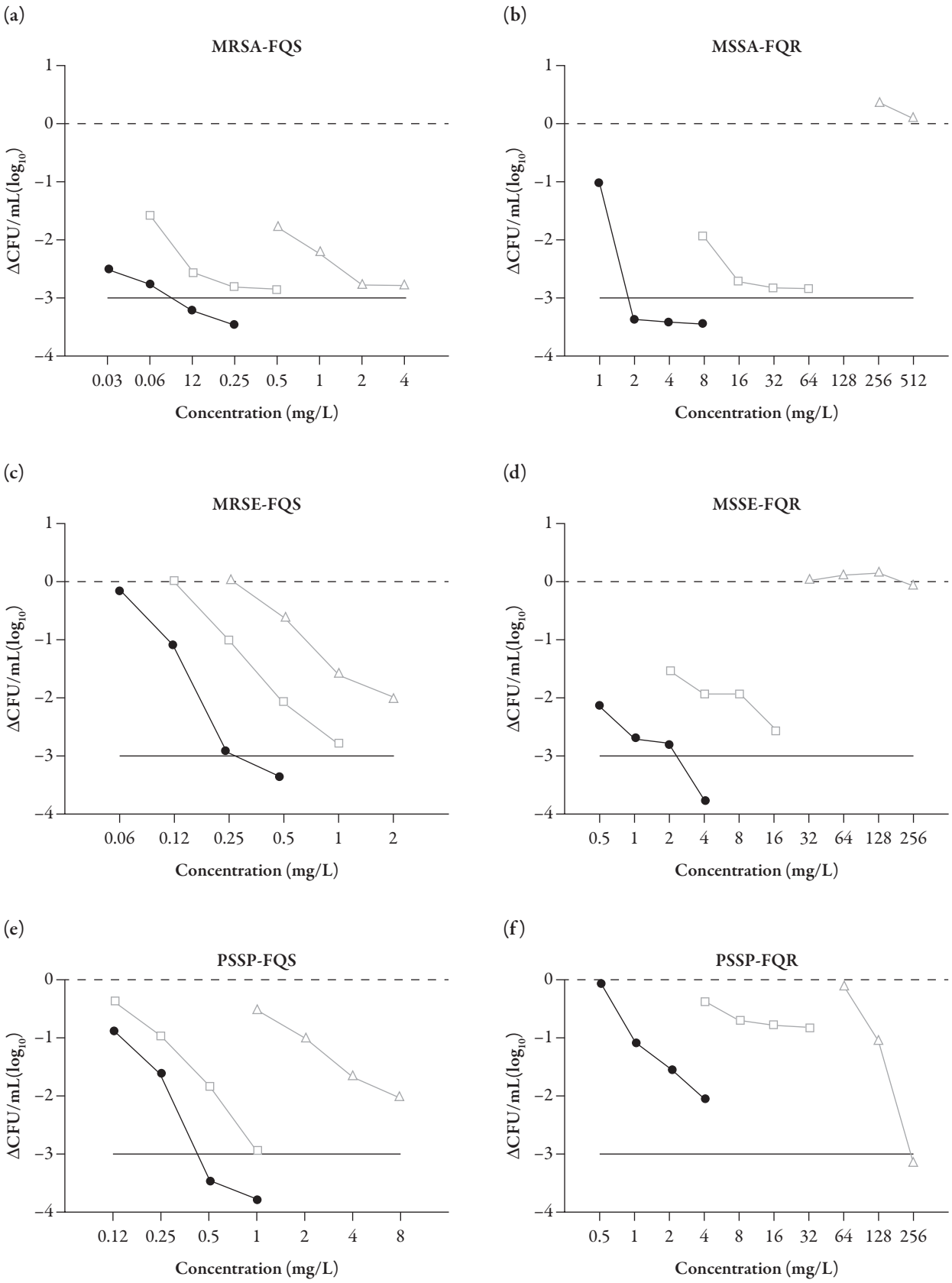
macrolides, or aminoglycosides [33]. Minimum bactericidal concentration (MBC) and MIC data indicated that, compared with ciprofloxacin, gatifloxacin, and moxifloxacin, besifloxacin was the most potent fluoroquinolone against *S. aureus*, *S. epidermidis*, and *S. pneumoniae*, with MBC:MIC ratios ≤4 for 97.5% of all the isolates tested [33]. Unlike comparator fluoroquinolones, besifloxacin maintained high potency and bactericidal activity against strains that contained multiple mutations in the genes encoding DNA gyrase and topoisomerase IV (Fig. 2) [33]. Time-kill studies further showed that besifloxacin was bactericidal for *S. aureus*, *S. epidermidis*, *S. pneumoniae,* and *H. influenzae* within 45–60 minutes; while moxifloxacin and gatifloxacin required 2 hours or longer to reach a ≥3-log decrease in viable cells [34]. A recent study investigated the impact on fluoroquinolone susceptibility of genetic mutation frequencies in *S. aureus* DNA gyrase, and topoisomerase IV [35]. For all fluoroquinolones evaluated (besifloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, levofloxacin), MIC values rose concomitantly with the number of resistance-relevant genetic mutations. Besifloxacin showed the smallest degree of increase compared with the other agents, possibly related to its dual mechanisms of action on both DNA gyrase and topoisomerase IV [35].

While genetic mutations in DNA gyrase or topoisomerase genes are largely responsible for bacterial resistance to fluoroquinolones, the presence of drug efflux pumps in both Gram-positive and Gram-negative organisms may be contributive. In general, newer fluoroquinolones have been shown to be less susceptible to efflux pump-mediated resistance than older compounds. A recent study confirmed that besifloxacin retained antibacterial efficacy against a range of wild-type and efflux pump mutant strains (*S. aureus, S. pneumoniae, Escherichia coli, H. influenzae, and Pseudomonas aeruginosa*), with MIC values ranging from 0.015–4 μg/mL. Those values were lower or similar to those of comparator fluoroquinolones (e.g., moxifloxacin, ciprofloxacin) [36].

Pharmacokinetic/Pharmacodynamic Data

In human pharmacokinetic and pharmacodynamic studies, high levels of besifloxacin were found in tear and conjunctival samples. Besifloxacin tear concentrations following a single topical dose remained higher than the MIC_{90} values for the common bacterial conjunctivitis pathogens (i.e., *S. aureus, S. pneumoniae, S. epidermidis*, and *H. influenzae*) for 24 hours or longer. Proksch et al. [37] calculated the maximum mean concentration (C_{max}) and area under the curve from the time of dosing to 24 hours ($AUC_{(0–24)}$) for besifloxacin in tears after a single dose. Then, using the MIC_{90} values for ocular pathogens known to cause acute bacterial conjunctivitis, the authors demonstrated that the C_{max}/MIC_{90} and $AUC_{(0–24)}/MIC_{90}0$ ratios were considerably greater than the target ratios currently used to predict clinical and microbial success for systemically administered antibiotics (i.e., C_{max}/MIC_{90} and $AUC_{(0–24)}/MIC_{90}$ target ratios for Gram-positive bacteria are ≥10 and ≥30–50, respectively, and both ratios ≥100–125 for Gram-negative bacteria). The observed elimination half-life of besifloxacin in human tears was 3.4 hours [26]. Similarly, conjunctival concentrations of besifloxacin have been shown to exceed the MIC_{90} values of MSSA and methicillin-sensitive *S. epidermidis* for at least 2.0 hours [38]. The mean residence time of besifloxacin in the conjunctiva was 4.7 hours, compared with 3.0 hours for moxifloxacin and 2.9 hours for gatifloxacin [38].

Aqueous humor concentrations of besifloxacin, moxifloxacin, and gatifloxacin were compared in human subjects undergoing


(a)
MRSA-FQS
ΔCFU/mL(log10)
1
0
−1
−2
−3
−4
0.03 0.06 12 0.25 0.5 1 2 4
Concentration (mg/L)
(b)
MSSA-FQR
ΔCFU/mL(log10)
1 2 4 8 16 32 64 128 256 512
Concentration (mg/L)
(c)
MRSE-FQS
ΔCFU/mL(log10)
0.06 0.12 0.25 0.5 1 2
Concentration (mg/L)
(d)
MSSE-FQR
ΔCFU/mL(log10)
0.5 1 2 4 8 16 32 64 128 256
Concentration (mg/L)
(e)
PSSP-FQS
ΔCFU/mL(log10)
0.12 0.25 0.5 1 2 4 8
Concentration (mg/L)
(f)
PSSP-FQR
ΔCFU/mL(log10)
0.5 1 2 4 8 16 32 64 128 256
Concentration (mg/L)

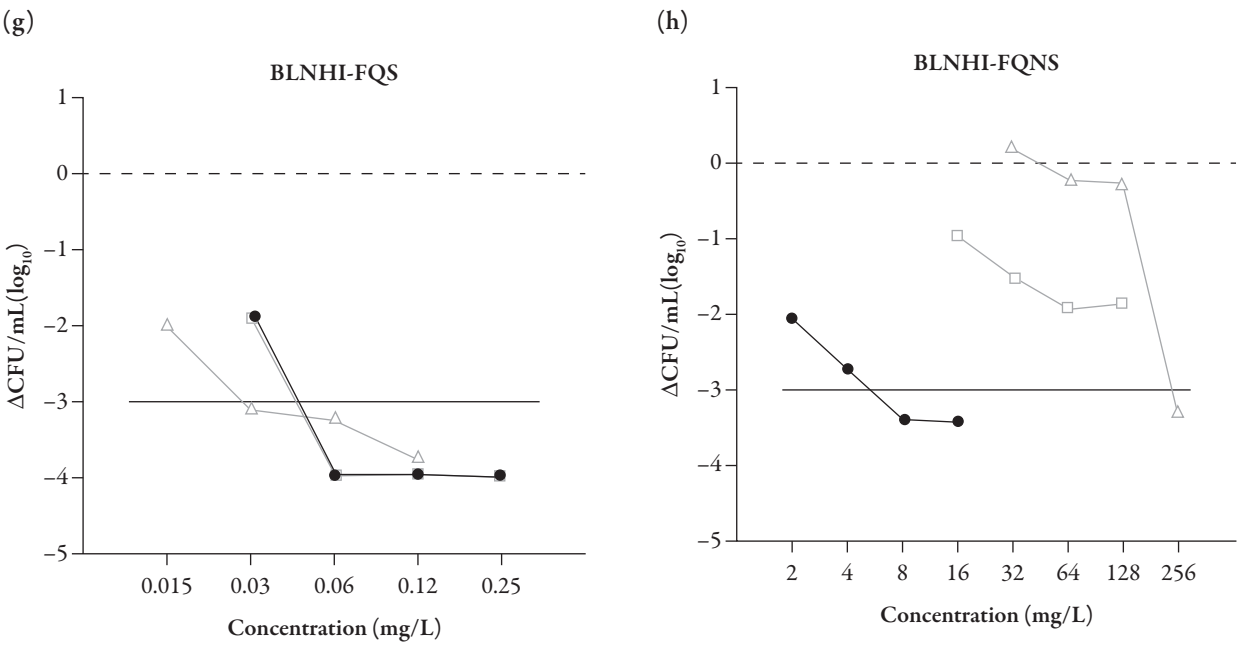


Fig. 2 Change in colony-forming units (CFU)/mL at 2 hours after the addition of 1×, 2×, 4×, or 8× the minimum inhibitory concentration of besifloxacin (filled circles), moxifloxacin (open squares), or ciprofloxacin (open triangles). Continuous horizontal line signifies a 3-log reduction in viable cells. (**a**) *MRSA-FQS* methicillin-resistant *Staphylococcus aureus*-fluoroquinolone susceptible, (**b**) *MSSA-FQR* methicillin-susceptible *Staphylococcus aureus*-fluoroquinolone resistant, (**c**) *MRSE-FQS* methicillin-resistant *Staphylococcus epidermidis*-fluoroquinolone susceptible, (**d**) *MSSE-FQR* methicillin-susceptible *Staphylococcus epidermidis*-fluoroquinolone resistant, (**e**) *PSSP-FQS* penicillin-susceptible *Streptococcus pneumoniae*-fluoroquinolone susceptible, (**f**) *PSSP-FQR*, penicillin-susceptible *Streptococcus pneumoniae*-fluoroquinolone resistant, (**g**) *BLNHI-FQS* β-lactamase-negative *Haemophilus influenzae*-fluoroquinolone susceptible, (**h**) *BLNHI-FQNS* β-lactamase-negative *Haemophilus influenzae*-fluoroquinolone nonsusceptible. *Reproduced with permission from Haas W, Pillar C, Hesje CK, Sanfilippo CM, Morris TW. Bactericidal activity of besifloxacin against staphylococci, Streptococcus pneumoniae and Haemophilus influenzae. J Antimicrob Chemother. 2010;65:1441–7*

uncomplicated cataract surgery 60 minutes after instillation of a single drop [39]. Mean aqueous humor levels were low for all three fluoroquinolones: besifloxacin (0.135 μg/mL), moxifloxacin (0.668 μg/mL), and gatifloxacin (0.125 μg/mL). Low aqueous humor levels were similarly observed in another study in which besifloxacin and moxifloxacin were dosed four times, one drop every 10 minutes beginning 1 hour prior to cataract surgery resulting in mean aqueous humor concentrations of moxifloxacin and besifloxacin were 1.6 μg/mL and 0.032 μg/mL, respectively [40]. The clinical relevance of fluoroquinolone aqueous humor penetration remains a subject of debate, and the pharmacokinetic data reported to date do not suggest adequate aqueous humor penetration of fluoroquinolones to have activity against the organisms most commonly isolated in postoperative endophthalmitis. However, the impact and interplay between MIC values and pharmacodynamic/pharmacokinetic parameters such as C_{max}, AUC, and percentage of time during which the concentration exceeds the MIC continue to be studied as possibly more accurate predictors of clinical efficacy than simple comparisons of MIC and tissue concentration [41].

In contrast to the high ocular surface concentrations, the average systemic

concentration of besifloxacin after repeated three-times daily (t.i.d.) dosing was found to be less than 0.5 ng/mL, suggesting an extremely low risk of systemic side effects after topical administration of besifloxacin [37]. Indeed, an analysis of the safety of besifloxacin ophthalmic suspension involving a total of 1,350 patients from two phase 1 safety studies, a phase 2 pharmacokinetic study, and three clinical trials demonstrated the favorable safety profile of besifloxacin [42].

Clinical Studies of Besifloxacin in the Treatment of Acute Bacterial Conjunctivitis

The clinical efficacy and safety of besifloxacin ophthalmic suspension 0.6% applied t.i.d. at approximately 6-hour intervals for 5 days was examined in three randomized, double-masked, vehicle- or comparator-controlled clinical studies in patients with culture-confirmed acute bacterial conjunctivitis [43–45]. Clinical resolution and microbial eradication were measured at similar timepoints in all three studies. Clinical resolution was defined as the absence of the two key clinical signs of acute conjunctivitis: conjunctival discharge and bulbar injection. Microbial eradication was defined as the absence on follow-up cultures of all ocular bacterial species that were identified at or above defined threshold levels in baseline cultures [43–45].

Karpecki et al. studied the clinical efficacy of besifloxacin ophthalmic suspension 0.6% in a parallel-group, vehicle-controlled clinical study that enrolled 269 patients with culture-confirmed acute bacterial conjunctivitis from 35 centers throughout the United States [44]. A significantly greater proportion of patients (44/60, 73.3%) in the besifloxacin-treated group achieved clinical resolution at the primary analysis timepoint (day 8 or 9) than in the vehicle-treated group (25/58 patients, 43.1%; $P < 0.001$). Microbial eradication at day 4 (± 1 day) was seen in a significantly higher proportion of patients in the besifloxacin (54/60, 90%) group than in the vehicle (27/58, 46.6%; $P < 0.001$) group. Microbial eradication at day 8 (or 9) also showed a similar significant difference (besifloxacin, 53/60, 88.3% vs. vehicle, 35/58, 60.3%; $P < 0.001$) [44]. The microbial eradication rate was significantly greater with besifloxacin than with vehicle for Gram-positive organisms [44].

A vehicle-controlled study by Tepedino et al. randomized 957 patients with acute conjunctivitis, of whom 390 were identified as having culture-confirmed acute bacterial conjunctivitis [43]. Consistent with the study by Karpecki et al., treatment with besifloxacin resulted in a significantly greater percentage of patients achieving clinical resolution at the primary analysis timepoint (day 5 ± 1 day) (90/199, 45.2%) than vehicle treatment (63/191, 33.0%; $P = 0.0084$) [44]. Similarly, a significantly greater percentage of patients attained clinical resolution at day 8 (168/199, 84.4%) with besifloxacin than with the vehicle alone (132/191, 69.1%; $P = 0.0011$) [43]. Significantly greater microbial eradication was achieved with besifloxacin than with the vehicle alone: 91.5% (182/199) of besifloxacin-treated patients achieved microbial eradication at the primary analysis timepoint (day 5 ± 1 day), compared to only 59.7% (114/191; $P < 0.0001$) of vehicle-treated patients. This significant difference in microbial eradication was also seen at day 8 or 9 (besifloxacin, 176/199, 88.4%, vs. vehicle, 137/191, 71.7%; $P < 0.0001$) [43]. At the primary analysis timepoint, besifloxacin-treated patients had a significantly higher microbial eradication rate than vehicle-treated patients ($P < 0.01$) for all pathogens except *S. epidermidis* [43].

McDonald et al. reported a noninferiority study that compared the safety and efficacy of besifloxacin ophthalmic suspension 0.6% and moxifloxacin ophthalmic solution 0.5%. Of the 1,161 patients randomized at 84 sites, 533 were confirmed to have acute bacterial conjunctivitis on baseline cultures [45]. Clinical resolution rates at the primary analysis timepoint (day 5) were not significantly different between besifloxacin-treated (147/252, 58.3%) and moxifloxacin-treated (167/281, 59.4%; $P = 0.6520$) patients. Microbial eradication rates at day 5 were also not significantly different between the besifloxacin (235/252, 93.3%) and moxifloxacin groups (256/281, 91.1%; $P = 0.1238$) [45]. The absence of significant differences in the clinical resolution and microbial eradication rates between the two fluoroquinolones continued at a later timepoint (day 8+1 day) [45]. Eradication rates for the organisms most commonly cultured in this study (*S. pneumoniae, H. influenzae, S. aureus,* and *S. epidermidis)* were similarly high for besifloxacin- and moxifloxacin-treated patients, with day 5 eradication rates ranging from 75.6–96.6% of patients [45]. Efficacy against Gram-positive organisms was similar between the two drugs, but at day 8, besifloxacin-treated patients had a significantly higher eradication rate against Gram-negative organisms (90.7%) compared with moxifloxacin-treated patients (83.8%; $P = 0.0375$) [45].

More recently, Silverstein et al. studied the safety and efficacy of besifloxacin ophthalmic suspension 0.6% administered twice daily (b.i.d.) for 3 days in a multicenter, randomized, parallel-group, vehicle-controlled study [46]. Of 202 patients (of whom 102 were ≤17 years of age) who were randomized to either besifloxacin or vehicle, 109 had culture-confirmed bacterial conjunctivitis. At visit 2 (day 4 or 5), rates of clinical resolution (37/53 [69.8%] vs. 21/56 [37.5%]; $P < 0.001$) and bacterial eradication (46/53 [86.8%] vs. 32/56 [57.1%]; $P < 0.001$) were significantly better in besifloxacin-treated patients [46]. Adherence to a drug treatment regimen is inversely proportional to dose number and timing [47, 48]. Furthermore, low adherence may result in poor clinical outcomes, increased

Table 2 Clinical resolution and microbial eradication rates (% of patients) at primary analysis visits in besifloxacin clinical studies

	Karpecki et al.[a] [44]	Tepedino et al.[a] [43]	McDonald et al.[a] [45]	Silverstein et al.[b] [46]
Primary outcome assessment	Visit 3 (day 8)	Visit 2 (day 5 ± 1)	Visit 2 (day 5)	Visit 2 (day 4 or 5)
Clinical resolution, % (*n*/*N*)				
Besifloxacin	73.3 (44/60)	45.2 (90/199)	58.3 (147/252)	69.8 (37/53)
Vehicle	43.1 (25/58)	33.0 (63/191)	—	37.5 (21/56)
Moxifloxacin	—	—	59.4 (167/281)	—
Microbial eradication, % (*n*/*N*)				
Besifloxacin	88.3 (53/60)	91.5 (182/199)	93.3 (235/252)	86.8 (46/53)
Vehicle	60.3 (35/58)	59.7 (114/191)	—	57.1 (32/56)
Moxifloxacin	—	—	91.1 (256/281)	—

[a] Besifloxacin administered thrice daily for 5 days
[b] Besifloxacin administered twice daily for 3 days

healthcare costs, and increased bacterial resistance levels due to suboptimal antibiotic concentrations [49]. Thus, the b.i.d. dose regimen of besifloxacin ophthalmic suspension 0.6% could contribute to better adherence, improved outcomes, and less potential for the development of bacterial resistance.

Table 2 [43–46] summarizes the efficacy results in the four clinical studies with besifloxacin ophthalmic suspension 0.6% administered t.i.d. or b.i.d., vehicle, and moxifloxacin ophthalmic solution 0.5%.

Clinical Safety Data

Besifloxacin ophthalmic suspension 0.6% was safe and well tolerated in each of the clinical studies. A pooled analysis of the adverse events reported across the three clinical studies in which besifloxacin ophthalmic suspension was administered t.i.d. for 5 days showed that the most commonly reported ocular adverse events in besifloxacin-treated patients were, in order of frequency, blurred vision (2.1%), eye pain (1.8%), eye irritation (1.4%), conjunctivitis (1.2%), and eye pruritus (1.1%) [50]. Besifloxacin-treated patients reported blurred vision, eye irritation, and conjunctivitis significantly less frequently than the patients receiving vehicle [50]. In addition, eye irritation was significantly less common for besifloxacin-treated eyes (0.3%) than for moxifloxacin-treated eyes (1.4%; $P = 0.02$) [45]. Table 3 lists the total ocular adverse events for the three clinical studies with t.i.d. dosing for each patient group [42].

In the clinical study in which besifloxacin ophthalmic suspension 0.6% was administered b.i.d. for 3 days, the treatment-emergent ocular adverse events reported in >1% of eyes ($n = 157$) treated with besifloxacin were

Table 3 Treatment-emergent ocular adverse events in ≥ 1%[a] of study eyes in any treatment group in clinical trials of besifloxacin administered three-times per day [42]

Adverse event, *n* (%)	Besifloxacin ophthalmic suspension 0.6% (*n* = 1,192)	Vehicle (*n* = 616)	Moxifloxacin ophthalmic solution 0.5% (*n* = 579)	*P*-value[b]
Total number of AEs	191	146	81	—
Number of eyes with ≥1 AE	139 (11.7)	101 (16.4)	54 (9.3)	0.006
Blurred vision	25 (2.1)	24 (3.9)	3 (0.5)	0.032
Eye irritation	17 (1.4)	18 (2.9)	8 (1.4)	0.046
Eye pain	22 (1.8)	11 (1.8)	7 (1.2)	>0.99
Conjunctivitis	14 (1.2)	15 (2.4)	5 (0.9)	0.049
Eye pruritus	13 (1.1)	10 (1.6)	2 (0.3)	0.38
Conjunctivitis, bacterial	7 (0.6)	9 (1.5)	2 (0.3)	0.068
Ocular AE of interest with incidence of 0.5–1.0% in any treatment group				
Punctate keratitis	4 (0.3)	2 (0.3)	3 (0.5)	>0.99

[a] Except where stated otherwise

[b] *P*-values based on Fisher's exact test comparing besifloxacin ophthalmic suspension 0.6% with vehicle

AEs adverse events

bacterial conjunctivitis (1.9% of treated eyes), conjunctivitis (1.9%), and allergic conjunctivitis (1.3%). Patients in the vehicle group (154 treated eyes) experienced higher rates of bacterial conjunctivitis (3.2% of treated eyes), conjunctivitis (2.6%), and instillation-site pain and corneal staining (1.3% for both versus 0.0% for both in the besifloxacin group). All ocular adverse events were classified as mild or moderately severe [46].

Headache was the only nonocular adverse event reported by besifloxacin-treated patients (1.8% of subjects) at a frequency of >1% in the t.i.d. clinical trials [50]. In the b.i.d. study, only 2.1% of patients receiving besifloxacin reported a nonocular adverse event, and none of those events were considered treatment related [46]. Serious nonocular treatment-related adverse events were not reported in any of the besifloxacin clinical studies [46, 50].

Besifloxacin Treatment in Pediatric Patients

A post hoc pediatric subgroup analysis was conducted with data from 815 pediatric patients (1–17 years of age) who participated in the clinical studies in which the besifloxacin dosage was t.i.d. [42]. Of the 815 pediatric patients, 447 had culture-confirmed, acute bacterial conjunctivitis. In the pediatric subgroup of the two vehicle-controlled trials, clinical resolution rates at visits 2 and 3 were significantly better for besifloxacin-treated patients than for vehicle-treated patients (visit 2: 53.7% vs. 41.3%; visit 3: 88.1% vs. 73.0%; $P < 0.05$ for both). Besifloxacin-treated patients also had significantly better microbial eradication rates than vehicle-treated patients at visits 2 (85.8% vs. 56.3%) and 3 (82.8% vs. 68.3%; $P < 0.05$ for both). The rates of clinical resolution and microbial eradication were similar in besifloxacin-treated and moxifloxacin-treated pediatric patients in the comparator trial [42]. Besifloxacin was well tolerated by pediatric patients, with adverse events occurring at similar rates among besifloxacin-, vehicle-, and moxifloxacin-treated patients [42].

Prevention and Treatment of Infections Following Ocular Surgery

Endophthalmitis has become increasingly resistant to older fluoroquinolones [11]. Two recent preclinical studies have shown the potential usefulness of besifloxacin for the prevention and treatment of this disease. In a rabbit model of penicillin-resistant *S. pneumoniae* endophthalmitis, besifloxacin was shown to be as effective as gatifloxacin and moxifloxacin in the prophylaxis and treatment of endophthalmitis. Eyes treated with a fluoroquinolone had significantly lower clinical scores at 24 hours after infection and bacteria recovered [as measured by log colony-forming units (CFU)/mL] from the aqueous humor than eyes treated with a phosphate-buffered solution (PBS) [51]. The effect of besifloxacin was also investigated in a rabbit model of MRSA-induced endophthalmitis [25]. Besifloxacin was found to significantly reduce the clinical signs of endophthalmitis compared with saline alone in the rabbit model of MRSA-induced endophthalmitis. Levofloxacin, moxifloxacin, and gatifloxacin did not produce significant clinical resolution of the clinical signs of endophthalmitis induced by MRSA in this model [25].

Similarly, two preclinical studies compared the use of besifloxacin for the treatment of MRSA keratitis with gatifloxacin and moxifloxacin [52, 53]. In a rabbit model of MRSA keratitis, the results of late treatment (16 hours after infection) with besifloxacin, gatifloxacin, moxifloxacin, and PBS were

compared [52]. Although no differences were seen in the clinical severity scores among the four groups, the mean log10 CFU count of MRSA recovered from the corneas was significantly less following besifloxacin treatment (5.111 ± 0.251) than after application of PBS (7.006 ± 0.144), gatifloxacin (7.108 ± 0.346), or moxifloxacin (7.473 ± 0.144; all $P < 0.001$). Thus, besifloxacin reduced the number of MRSA CFU in the rabbit cornea significantly more than gatifloxacin and moxifloxacin when given 16 hours after infection. The MIC for MRSA was eightfold lower for besifloxacin than for moxifloxacin and gatifloxacin [52]. In a more recent study involving an early treatment model (10 hours after infection) of MRSA keratitis, infected rabbit eyes were treated with PBS, besifloxacin, gatifloxacin, or moxifloxacin. The mean log10 CFU count from the cornea 10 to 18 hours after infection was significantly lower following besifloxacin treatment (3.57 ± 0.38) than after treatment with gatifloxacin (5.65 ± 0.40), moxifloxacin (6.24 ± 0.39), or PBS (7.39 ± 0.10; all $P < 0.001$). Reductions in clinical severity scores were similar for all three fluoroquinolones and were significantly less for all three than with PBS. Besifloxacin also had an MIC for MRSA that was eightfold lower than the MICs for gatifloxacin and moxifloxacin [53].

The efficacy of gatifloxacin, moxifloxacin, besifloxacin, and PBS against two strains of *P. aeruginosa* – one susceptible to and one resistant to ciprofloxacin and levofloxacin – was compared in rabbit corneas injected with 10^3 CFU of *P. aeruginosa*, one of the most frequently isolated bacterium from people with keratitis [54]. The fluoroquinolones were administered between 16 and 24 hours after infection. For corneas infected with the susceptible strains of *P.* aeruginosa, no significant differences were seen in the reduction of clinical severity scores at 25 hours postinfection among the three fluoroquinolones or between the fluoroquinolones and PBS. Among the resistant strains, the clinical severity score at 25 hours was significantly less with besifloxacin than with moxifloxacin, but not significantly different from gatifloxacin or PBS. All three fluoroquinolones had significantly lower mean log10 CFU counts of susceptible bacteria recovered from the rabbit corneas at 25 hours postinfection than PBS, and no significant differences among these agents were observed. Among the resistant strains, a significantly lower mean log10 CFU count of bacteria was recovered with besifloxacin (3.16 ± 1.88) at 25 hours than with gatifloxacin (4.994 ± 1.21) or moxifloxacin (5.35 ± 1.47). The three active treatments all had significantly lower mean log10 CFU count of resistant bacteria recovered than PBS (8.07 ± 1.14). The MICs against the susceptible strain were 0.5 μg/mL for both besifloxacin and moxifloxacin, and 0.25 μg/mL for gatifloxacin, while the MICs against the resistant strain were 2 μg/mL for besifloxacin, 16 μg/mL for gatifloxacin, and 32 μg/mL for moxifloxacin [54].

A recent human case report described the successful use of besifloxacin to treat a corneal ulcer, presumably of *P. aeruginosa* etiology, in a contact lens wearer [55]. Bulbar conjunctiva on the affected eye demonstrated severe hyperemia (grade 3+) with an injection area and grade 1+ chemosis. A grade 3 reaction was evident in the anterior chamber. A ring infiltrate surrounding the corneal lesion was strongly suggestive of *P. aeruginosa*. Initial therapy consisted of besifloxacin drops given hourly during waking hours on the first day, every 3 hours overnight, and supplemented by ciprofloxacin ointment at bedtime. With continued improvement noted on each subsequent treatment day, besifloxacin dosage was tapered every 48 hours by lengthening

administration intervals, first to every 2 hours and eventually to four times per day. Complete resolution was noted approximately 1 month after treatment initiation.

As mentioned previously, aqueous humor concentrations of besifloxacin and other topical fluoroquinolones are generally low, raising doubts about their usefulness for preventing or treating postsurgical endophthalmitis. It has been argued that efficient eradication of bacterial pathogens on the ocular surface, thus preventing entry into the eye altogether, may represent the most rational approach to dealing with this problem [39]. To that end, besifloxacin has demonstrated longer mean residence time in conjunctival tissue and a greater AUC/MIC ratio than gatifloxacin and moxifloxacin against resistant organisms on the ocular surface [38]. Furthermore, while these data are promising, the relevance of animal models of endophthalmitis and keratitis to human disease has not been established. Clearly, more clinical experience will be needed in these areas.

CONCLUSION

Besifloxacin is a chloro-fluoroquinolone developed solely for topical ophthalmic use that is associated with greater in-vitro potency relative to other fluoroquinolones, particularly among MRSA and MRSE species. Microbiological studies have shown that besifloxacin has relatively high potency against staphylococcal isolates resistant to other fluoroquinolones and was rapidly bactericidal against all the pathogens associated with bacterial conjunctivitis. Besifloxacin has also exhibited balanced dual-targeting of the two enzymes essential to bacterial replication. The use of besifloxacin for ocular infections theoretically reduces the risk for development of resistance, although the possibility of cross-resistance from other fluoroquinolones remains. The three controlled clinical studies of besifloxacin t.i.d. for 5 days and a study of besifloxacin b.i.d. for 3 days found that besifloxacin effectively resolved the signs of acute bacterial conjunctivitis and eradicated the causative pathogens. The safety and tolerability of besifloxacin were similar to those of vehicle. Treatment-emergent ocular and nonocular adverse events occurred at similar rates among besifloxacin- and moxifloxacin-treated patients, except that besifloxacin-treated patients reported eye irritation less frequently than did moxifloxacin-treated patients. Besifloxacin was safe and effective in the treatment of bacterial conjunctivitis in the pediatric population from age 1 year through adolescence. Furthermore, besifloxacin has been shown in preclinical animal studies to be potentially effective for the prevention and treatment of infections following ocular surgery.

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