Eye (2007) 21, 990–994

www.nature.com/eye

LABORATORY STUDY

Penetration of second-, third-, and fourth-generation topical fluoroquinolone into aqueous and vitreous humour in a rabbit endophthalmitis model

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Received: 9 January 2006
Accepted in revised form: 2 April 2006
Published online: 26 May 2006

Abstract

Aims **This study was designed to investigate the penetration of second-, third- and fourth-generation topical fluoroquinolone into aqueous and vitreous humour in a rabbit endophthalmitis model.**

Methods **Thirty New Zealand white rabbits were divided into six groups. Left eye was infected with an intravitreal inoculum of *Staphylococcus aureus*. Groups 1, 2, 3, 4, and 5 received topical ofloxacin, ciprofloxacin, lomefloxacin, levofloxacin, or moxifloxacin treatment 24 h after the inoculation, respectively. No treatment was given to group 6 as the control group ($n=5$). Aqueous and vitreous samples were obtained 30 min after the last drop. High-performance liquid chromatography was used to determine the fluoroquinolone concentration.**

Results **In the normal and inflamed eyes, mean aqueous concentrations of ofloxacin were 1.90 and 2.69 μg/ml, ciprofloxacin were 2.16 and 3.65 μg/ml, lomefloxacin were 3.54 and 1.19 μg/ml, levofloxacin were 2.89 and 9.41 μg/ml, and moxifloxacin were 4.92 and 43.33 μg/ml, respectively. Mean vitreous concentrations of ofloxacin were 0.25 and 0.07 μg/ml, ciprofloxacin were 0.08 and 0.32 μg/ml, lomefloxacin were 0.001 and 0.03 μg/ml, levofloxacin were 0.03 and 0.09 μg/ml, and moxifloxacin were 0.28 and 2.68 μg/ml, in normal and inflamed eyes, respectively. Moxifloxacin achieved a significantly higher concentration in aqueous and vitreous humour of infected eyes compared with ofloxacin ($P<0.01$), ciprofloxacin ($P<0.05$), lomefloxacin ($P<0.01$), and levofloxacin ($P<0.05$).**

Conclusion **This study demonstrated that fourth-generation fluoroquinolone, moxifloxacin, seems to have better penetration to inflamed ocular tissues in rabbit.**

Eye (2007) **21,** 990–994; doi:10.1038/sj.eye.6702414; published online 26 May 2006

Keywords: topical fluoroquinolones; penetration; inflamed eye

Introduction

Superficial and deep ocular infections, such as conjunctivitis, corneal ulcers, and endophthalmitis, are caused by a diverse group of bacterial, viral, and fungal pathogens. Bacterial endophthalmitis most commonly occurs as a postoperative complication, but it may also result from penetrating ocular trauma or systemic infection.[1,2] *Staphylococcus epidermidis* and *Staphylococcus aureus* are important causes of postoperative bacterial endophthalmitis.[3] Topical fluoroquinolones are commonly used antibacterials in the treatment of ocular infectious diseases.[4]

The first quinolone, nalidixic acid, was introduced in 1962. Since then, structural modifications have resulted in the development

of second-, third-, and fourth-generation fluoroquinolones based on their spectrum of antimicrobial activity classification. Quinolones rapidly inhibit DNA synthesis by promoting cleavage of bacterial DNA in the DNA–enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death.[5,6] The second generation (ofloxacin, ciprofloxacin, lomefloxacin) has expanded Gram-negative and atypical pathogen coverage, but limited the antimicrobial activity against Gram-positive bacteria. The third generation (levofloxacin) has increased Gram-positive coverage and retained antibacterial activity against Gram-negative and atypical bacteria. The fourth generation (moxifloxacin) has improved Gram-positive coverage, gained anaerobic coverage, and maintained Gram-negative activity.[7]

Although there are several studies related to the penetration of second- and third-generation fluoroquinolones into ocular tissues,[8–13] reports on penetration of these antimicrobials into the inflamed eyes are limited.[14,15] Furthermore, to the best of our knowledge, there are no data on penetration of topical administration of levofloxacin and moxifloxacin in inflamed eyes. The aim of the study was to evaluate the aqueous and vitreous ofloxacin, ciprofloxacin, lomefloxacin, levofloxacin, and moxifloxacin concentrations following topical instillation in normal and inflamed eyes, and to compare the intraocular concentrations.

Materials and methods

Thirty New Zealand white rabbits weighing between 2.1 and 3 kg were randomized into six groups. Throughout the study, all animals were maintained in accordance with the Association for Research in Vision and Ophthalmology statement for use of animals in ophthalmic and vision research.

Endophthalmitis model

Mydriasis was achieved with phenylephrine hydrochloride (2.5%) and atropine sulphate (1%) ophthalmic solution before indirect ophthalmoscopy. All rabbits were anaesthetized via intramuscular injection of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (50 mg/kg). Before the inoculation of the microorganism into the vitreous cavity, animals underwent a complete baseline eye examination that included slit-lamp biomicroscopy and indirect ophthalmoscopy to detect abnormal pathologic findings such as inflammation. Under direct observation, all intravitreal injections were made with a 27-G needle attached to a 1-ml tuberculin syringe approximately 2 mm from the superonasal limbus positioned in the midvitreous cavity, with the bevel directed away from the retina. All intravitreal injections were given to the left eyes; the right eye of each animal was used as an uninoculated intact eye. *S. aureus* (ATCC® 29213) was used to induce intraocular infection.

Fluoroquinolone treatment

The animals were divided equally and randomly into six groups. Twenty-four hours after the inoculation, two drops of ofloxacin 0.3%, ciprofloxacin 0.3%, lomefloxacin 0.3%, levofloxacin 0.5%, and moxifloxacin 0.5% were instilled in the eyes of the animals in the groups 1, 2, 3, 4, 5, respectively, every 30 min for 3 h and every 60 min for the following 3 h. No treatment was applied to group 6 as it was the control group. Then, 30 min after the last drop of fluoroquinolone, aqueous and vitreous samples were taken and stored at -80°C until analysis.

High-performance liquid chromatography (HPLC) analysis of fluoroquinolones

Fluoroquinolone levels in the aqueous and vitreous samples were measured by using HPLC. Concentrations of fluoroquinolones in aqueous and vitreous humour were determined by HPLC as defined previously.[16,17] The separation was performed on a Radial-pak, C18 cartridge column (100×8 mm i.d., particle size 10 μm; Waters, Milford, MA, USA) and precolumn (C18). The sample elution was monitored on fluorescence detector using excitation and emission wavelengths of 280 and 455 nm, respectively. The mobile phase consisted of acetonitrile and 0.1 M NaH_2PO_4 (2 : 8 (v/v); pH = 3.9). The mobile phase was delivered at a flow rate of 2 ml/min. Quinine sulphate was used as an internal standard (2 μg/ml). Aqueous and vitreous humour samples were directly injected after dilution and addition of quinine sulphate. Twenty microlitres of aliquot was injected through the column. The within-day and day-to-day precision values were less than 9% for fluoroquinolones at 0.05, 0.1, and 0.4 μg/ml ($n = 6$), the within-day and day-to-day accuracy values were in the range of 96.33–102.67% for fluoroquinolones at the concentrations given above, and the detection limit corresponding to signal-to-noise ratio of 3 : 1 was 0.6 ng/ml.[18,19]

Statistics

Data were expressed as mean, SEM, and range. Concentrations of fluoroquinolones in normal and infected ocular tissues were compared with Kruskal–Wallis test, a nonparametric test for the paired samples. A P-value ≤ 0.05 was accepted as statistically significant.

Table 1 Aqueous humour and vitreous humour concentrations of ofloxacin, ciprofloxacin, lomefloxacin, levofloxacin, and moxifloxacin in the normal and infected eyes of rabbits

	Ofloxacin		*Ciprofloxacin*		*Lomefloxacin*		*Levofloxacin*		*Moxifloxacin*	
	Normal	*Infected*	*Normal*	*Infected*	*Normal*	*Infected*	*Normal*	*Infected*	*Normal*	*Infected*
Aqueous Mean concentration (SEM) (μg/ml)	1.90 (0.12)	2.69 (0.74)	2.16 (0.58)	3.65 (0.96)	3.54 (2.14)	1.19 (0.12)	2.89 (1.30)	9.41 (2.02)	4.92 (0.32)	43.3 (21.47)
Vitreous Mean concentration (SEM) (μg/ml)	0.25 (0.08)	0.07 (0.02)	0.08 (0.01)	0.32 (0.17)	0.0013 (0.0002)	0.03 (0.01)	0.03 (0.03)	0.09 (0.05)	0.28 (0.14)	2.68 (2.12)

SEM: standard error of the mean.

Results

The concentrations of ofloxacin, ciprofloxacin, lomefloxacin, levofloxacin, and moxifloxacin of normal and inflamed eyes in the aqueous and vitreous humour are given in Table 1. In uninfected eyes, moxifloxacin penetrated the aqueous humour at significantly higher levels compared to ofloxacin ($P<0.01$), ciprofloxacin ($P<0.05$), and levofloxacin ($P<0.05$) and the vitreous humour at significantly higher levels compared to lomefloxacin ($P<0.01$) and levofloxacin ($P<0.01$). In infected eyes, moxifloxacin penetrated the aqueous humour at significantly higher levels than ofloxacin ($P<0.01$), ciprofloxacin ($P<0.01$), lomefloxacin ($P<0.01$), and levofloxacin ($P<0.05$), and the vitreous humour at significantly higher levels than ofloxacin ($P<0.01$), ciprofloxacin ($P<0.05$), lomefloxacin ($P<0.01$), and levofloxacin ($P<0.01$). Inflammation significantly increased the aqueous penetration of moxifloxacin ($P<0.01$) and levofloxacin ($P<0.01$). Vitreous penetration of ciprofloxacin ($P<0.05$) and moxifloxacin ($P<0.05$) also increased with inflammation significantly.

Discussion

Recent reports indicate that resistance to earlier-generation ocular antibiotics among clinical bacterial isolates is becoming more prevalent.[16,20,21] The increasing number of ocular surgical procedures poses a greater risk for perioperative infection. There is an upward trend in the incidence of bacterial infections after cataract surgery.[17,22] This increased risk of surgical complications such as postoperative endophthalmitis and keratitis.[20,23]

Emerging resistance of ocular pathogens to the older-generation fluoroquinolones, ciprofloxacin and ofloxacin, led to the development of the fourth-generation compounds, gatifloxacin and moxifloxacin. The alterations to the structure of the older-generation fluoroquinolones improve the spectrum of activity of fourth-generation fluoroquinolones to include strains of *Staphylococcus* and *Streptococcus* and species otherwise resistant to older-generation fluoroquinolones.[23,24] These new fourth-generation fluoroquinolones are particularly needed because the incidence of endophthalmitis is on the rise,[22,25] especially after clear corneal incision cataract surgery.[22,26] It has been demonstrated that perioperative treatment with a prior-generation fluoroquinolone is efficacious in eliminating bacteria found in the external ocular adnexa, the most likely source of the inoculum of bacteria during cataract surgery.[27]

The current experimental study demonstrated that moxifloxacin penetrated into the aqueous and vitreous humour better than ofloxacin, ciprofloxacin, lomefloxacin, and levofloxacin in the inflamed eyes. Inflammation increased the aqueous and vitreous penetration of moxifloxacin significantly. Moxifloxacin ophthalmic solution provided drug penetration at aqueous and vitreous concentrations greater than the minimum inhibitory concentrations for *S. epidermidis*, *Streptococcus pneumoniae*, veridans streptococci, enterococci, and Bacillus species, fluoroquinolone-susceptible *S. aureus* except fluoroquinolone-resistant *S. aureus* in normal eyes. In the infected eyes, moxifloxacin had drug penetration at aqueous and vitreous concentrations greater than these organisms as well as fluoroquinolone-resistant *S. aureus*.[23] On the other hand, it is not known why lomefloxacin demonstrated less penetration in inflamed eyes compared to healthy eyes, but our results would suggest that other quinolones be preferred in inflamed eyes.

Topical use of antibiotics in ophthalmology should have some advantages. There is a reduced risk of side effects and of resistance to applied antimicrobial drugs. Furthermore, for topical application loading with antibiotics is somewhat lower and their concentration in aqueous humour is often higher than after systemic

administration. Generally however, penetration of topically applied drugs into the anterior chamber is the limiting factor for their use. Drug penetration is limited by the physiological barrier of hydrophilic corneal stroma and lipophilic epithelium and endothelium. The corneal epithelium contains tight intercellular junctions. Therefore, to penetrate into the anterior chamber, the antibiotics must diffuse directly through a hydrophobic epithelium. Thus, lipid-soluble drugs tend to pass more rapidly through the epithelium. Moxifloxacin, which is formulated approximately at a pH of 7 is soluble in the tear film, which has a pH of 7.2–7.4. To penetrate into the anterior chamber, agents must also pass through the hydrophilic corneal stroma. Therefore, topically applied drugs must be both water and lipid soluble to reach high aqueous humour concentrations.[28] In addition to the pharmaceutical agents being able to achieve therapeutic intracameral levels, they must be efficacious against the target organisms, with a broad spectrum of antimicrobial activity and a decreased level of resistance. The fourth generation of fluoroquinolones is believed to be such agents.

The current study presents assessments of fluoroquinolones penetration from aqueous and vitreous humour samples in infected rabbit eye. Moxifloxacin had better aqueous and vitreous penetration than that of older-generation fluoroquinolones. Moxifloxacin also exceeded the known MIC values for most pathogens that cause endophthalmitis in infected rabbit eye. Hariprasad *et al*[13] found that aqueous and vitreous concentrations achieved in uninfected human eyes after topical administration of moxifloxacin every 2 h were less than those found in this study in rabbit eyes. Whether the same would be the case with infected eyes needs to be studied. It is not known as to how much these results are applicable to humans. Further clinical studies are warranted to determine the aqueous and vitreous levels of fourth-generation fluoroquinolones in humans.

References

1 Puliafito CA, Baker AS, Haaf J, Foster CS. Infectious endophthalmitis: review of 36 cases. *Ophthalmology* 1982; **89**: 921–929.
2 Shrader SK, Band JD, Lauter CB, Murphy P. The clinical spectrum of endophthalmitis: incidence, predisposing factors, and features influencing outcome. *J Infect Dis* 1990; **162**: 115–120.
3 Endophthalmitis Vitrectomy Study Group. Results of the endophthalmitis vitrectomy study. A randomized trial of immediate vitrectomy and intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. *Arch Ophthamol* 1995; **113**: 1479–1496.
4 Leeming JP. Treatment of ocular infections with topical antibacterials. *Clin Pharmacokinet* 1999; **37**: 351–360.
5 Blondeau JM. Fluoroquinolones: mechanism of action, classification, and development of resistance. *Surv Ophthalmol* 2004; **49**: 73–78.
6 Hooper DC. Mode of action of fluoroquinolones. *Drugs* 1999; **58**: 6–10.
7 Ambrose PG, Owens RC, Quintiliani R, Nightingale CH. New generations of quinolones: with particular attention to levofloxacin. *Conn Med* 1997; **61**: 269–272.
8 Cekic O, Batman C, Yasar U, Basci NE, Bozkurt A, Kayaalp SO. Comparison of aqueous humour and vitreous humour levels of two 0.3% ciprofloxacin eyedrops. *Can J Ophthalmol* 1998; **33**: 373–376.
9 Çekiç O, Batman C, Yaşar U, Basci NE, Bozkurt A, Kayaalp SO. Human aqueous and vitreous humour levels of ciprofloxacin following oral and topical administration. *Eye* 1999; **13**: 555–558.
10 Yalvac IS, Basci NE, Bozkurt A, Duman S. Penetration of topically applied ciprofloxacin and ofloxacin into the aqueous humor and vitreous. *J Cataract Refract Surg* 2003; **29**: 487–491.
11 Yamada M, Mochizuki H, Yamada K, Kawai M, Mashima Y. Aqueous humor levels of topically applied levofloxacin, norfloxacin, and lomefloxacin in the same human eyes. *J Cataract Refract Surg* 2003; **29**: 1771–1775.
12 Solomon R, Donnenfeld ED, Perry HD, Snyder RW, Nedrud C, Stein J *et al.* Penetration of topically applied gatifloxacin 0.3%, moxifloxacin 0.5%, and ciprofloxacin 0.3% into the aqueous humor. *Ophthalmology* 2005; **112**: 466–469.
13 Hariprasad SM, Blinder KJ, Shah GK, Apte RS, Rosenblatt B, Holekamp NM *et al.* Penetration pharmacokinetics of topically administered 0.5% moxifloxacin ophthalmic solution in human aqueous and vitreous. *Arch Ophthalmol* 2005; **123**: 39–44.
14 Ozturk F, Kurt E, Inan UU, Kortunay MC, Ilker SS, Basci NE *et al.* Penetration of topical and oral ofloxacin into the aqueous and vitreous humor of inflamed rabbit eyes. *Int J Pharm* 2000; **204**: 91–95.
15 Bronner S, Jehl F, Peter JD, Ploy MC, Renault C, Arvis P *et al.* Moxifloxacin efficacy and vitreous penetration in a rabbit model of *Staphylococcus aureus* endophthalmitis and effect on gene expression of leucotoxins and virulence regulator factors. *Antimicrob Agents Chemother* 2003; **47**: 1621–1629.
16 Marangon FB, Miller D, Muallem MS, Romano AC, Alfonso EC. Ciprofloxacin and levofloxacin resistance among methicillin-sensitive *Staphylococcus aureus* isolates from keratitis and conjunctivitis. *Am J Ophthalmol* 2004; **137**: 453–458.
17 Colleaux KM, Hamilton WK. Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. *Can J Ophthalmol* 2000; **35**: 373–378.
18 Jim LK, el-Sayed N, Al-Khamis KI. A simple high performance liquid chromatographic assay for ciprofloxacin in human serum. *J Clin Pharm Ther* 1992; **17**: 111–115.
19 Basci NE, Bozkurt A, Kalayci D, Kayaalp SO. Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor. *J Pharm Biomed Anal* 1996; **14**: 353–356.
20 Hwang DG. Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. *Surv Ophthalmol* 2004; **49**: 79–83.
21 Mah FS. Fourth-generation fluoroquinolones: new topical agents in the war on ocular bacterial infections. *Curr Opin Ophthalmol* 2004; **15**: 316–320.

22 Nagaki Y, Hayasaka S, Kadoi C, Matsumoto M, Yanagisawa S, Watanabe K *et al*. Bacterial endophthalmitis alter small-incision cataract surgery: effect of incision placement and intraocular lens type. *J Cataract Refract Surg* 2003; **29**: 20–26.
23 Mather R, Karenchak LM, Romanowski EG, Kowalski RP. Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. *Am J Ophthalmol* 2002; **133**: 463–466.
24 Fukuda H, Kishii R, Takei M, Hayashi K, Hosaka M. Contributions of the 8-methoxy group of gatifloxacin to resistance selectivity, target preference, and antibacterial activity against *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2001; **45**: 1649–1653.
25 Kattan HM, Flynn Jr HW, Pflugfelder SC, Robertson C, Forster RK. Nosocomial endophthalmitis survey. Current incidence of infection after intraocular surgery. *Ophthalmology* 1991; **98**: 227–238.
26 Colleaux KM, Hamilton WK. Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. *Can J Ophthalmol* 2000; **35**: 373–378.
27 Kirsch LS, Jackson WB, Goldstein DA, Discepola MJ. Perioperative ofloxacin *vs* tobramycin: efficacy in external ocular adnexal sterilization and anterior chamber penetration. *Can J Ophthalmol* 1995; **30**: 11–20.
28 Price Jr FW, Dobbins K, Zeh W. Penetration of topically administered ofloxacin and trimethoprim into aqueous humor. *J Ocul Pharmacol Ther* 2002; **18**: 445–453.