Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-006-0241-9

CONCISE ARTICLE

Penetration of levofloxacin into the anterior chamber (aqueous humour) of the human eye after intravenous administration

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Abstract In the study presented here, levofloxacin concentrations in serum samples and the aqueous humour (AH) of 16 patients undergoing cataract extraction were measured in order to determine the penetration characteristics of levofloxacin into the AH of the non-inflamed human eye. Cataract removal was performed at various times (from 90 to 270 min) after the end of a 30-min intravenous infusion of 500 mg of levofloxacin. Serum samples were obtained 1 h after the end of levofloxacin administration (C_{max}); AH and a second serum sample were taken simultaneously during the operation, and the concentrations of levofloxacin in AH (C_{AH}) and serum (C_S) were determined using a rapid high-performance liquid chromatography assay. The mean C_{max} was 6.07 μg/ml (range 3.75–9.53 μg/ml, SD 1.83). The mean C_{AH} at the first hour following levofloxacin administration was 1.37 μg/ml (range 1.17–1.6 μg/ml, SD 0.22) and the mean ratio (R=C_{AH}/C_S) was 0.26 (range 0.24–0.3, SD 0.02). The mean C_{AH} at 125–270 min following levofloxacin administration was 1.39 μg/ml (range 0.82–1.98 μg/ml, SD 0.33) and the mean R was 0.3 (range 0.15–0.53, SD 0.11). Of 16 patients, 15 had a C_{AH} of >1 μg/ml 1 h after levofloxacin administration. In conclusion, 1 h after administration of 500 mg of levofloxacin, the levels obtained were higher than the MIC at which 90% of methicillin-susceptible *Staphylococcus aureus* and certain gram-negative bacteria strains are inhibited.

The abstract and preliminary results of this study were presented at the 7th European Congress of Chemotherapy and Infection, October 2005, Florence, Italy.

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Introduction

Penetration of antibiotics into the eye is variable [1], since the anterior chamber and the vitreous are avascular and the latter is also isolated from systemic circulation by the blood-ocular barrier. Previous studies documented that fluoroquinolones could enter the human eye after oral administration, but they also suggested that the concentrations in both the aqueous humour (AH) and the vitreous in a number of patients were lower than the minimum inhibitory concentrations (MIC) at which 90% of isolates are inhibited for common intraocular bacterial pathogens. Most of these prior studies were performed with ciprofloxacin [2–6].

Levofloxacin is a fluoroquinolone antibiotic [7] characterized by a broad antimicrobial spectrum that covers, among other organisms, some of the pathogens most

frequently responsible for acute endophthalmitis (methicillin-susceptible *Staphylococcus aureus*) and other sporadic agents of acute postoperative eye infections, such as *Escherichia coli* and other gram-negative rods. Orally administered levofloxacin is virtually completely bioavailable. Its elimination half-life allows dosing once or twice per day. Maximum concentrations in plasma (C_{max}) are achieved 1–2 h after an oral dose of the drug. It has low serum plasma protein binding (approximately 30%) and is freely distributed to well-perfused tissues. The volume of distribution is 1.4 l/kg. Previous pharmacokinetic studies documented that levofloxacin could adequately penetrate the cerebrospinal fluid (CSF) in the presence of meningeal inflammation both in animals and in humans. It can also penetrate the CSF in patients with minimal alteration of the blood-brain barrier (e.g. hydrocephalic patients with external ventriculostomies) [8].

This study was designed to determine the penetration characteristics of levofloxacin into the AH of the non-inflamed human eye.

Patients and methods

A total of 16 patients who underwent cataract extraction during the period from November to December 2003 were included. Cataract removal was performed at various times (from 90 to 270 min) after the end of a 30 min intravenous infusion of 500 mg of levofloxacin (as recommended by the manufacturer). The clinical study was approved by the Institutional Review Board at Hospital Clinic (Barcelona). All patients gave informed consent to take part in this study. Exclusion criteria were all other ocular diseases, previous ocular surgery, topical or systemic antibiotic treatment 1 week preoperatively and renal function impairment (creatinine clearance of <50 ml/min).

Serum samples were obtained 1 h after the end of levofloxacin administration (C_{max}). The AH sample and a second serum sample were taken simultaneously during surgery. Ocular paracentesis was performed and 0.05–0.1 ml of AH was aspirated. All samples were stored immediately at −80°C until processing. Details concerning patients and methods have been described previously.

Concentrations of levofloxacin were determined using a rapid high-performance liquid chromatography assay. The method was developed, validated and applied for the quantification of the drug in human plasma at the pharmacology laboratory of our hospital. The technique was designed according to previous data [9]. Samples were prepared by mixing 200 µl of plasma with a solution of perchloric acid and 50 µl of the clear supernatant was injected; 25 µl of AH sample was injected directly. The high-performance liquid chromatography analysis used a reverse-phase C18 column and a mobile phase consisting of a mixture of 35% acetonitrile, 65% 20 mM citric acid, 10 mM tetrabutylammonium acetate and 10 mM sodium dodecyl sulfate (pH 3.0). The fluorescence monitoring for levofloxacin was performed at an excitation wavelength of 297 nm and at an emission wavelength of 500 nm. The quantification limit was 0.05 µg/ml. The retention time for levofloxacin was 4.7 min. The accuracy of the method ranged from 94.1 to 104.9%, and the precision values ranged from 0.6 to 3.2% for intra-day precision and from 1.2 to 2.2% for the inter-day values. Standards (calibrators and quality controls) were prepared in human plasma for the levofloxacin plasma determination and in HPLC water for the AH determination.

Several data were analysed, including the mean maximum concentrations in plasma (C_{max}) and the mean concentrations in AH (C_{AH}) and serum (C_s) for samples taken simultaneously from patients who underwent surgery <125 min and 125–270 min following levofloxacin administration (the cut-off time was established according to data about maximum plasma concentrations following intravenously administered levofloxacin. The mean ratio of C_{AH} to C_s (R=C_{AH}/C_S) was calculated. The ratio informs about the average AH-to-plasma concentrations of levofloxacin and about penetration of the drug into the AH of the non-inflamed human eye.

Descriptive statistical analysis was done using the SPSS version 9.0 package (a statistical and data management program for Windows).

Results and discussion

Of 16 patients, nine were female and seven were male. The mean age was 77 years (range 61–93 years, SD 9.29). The mean weight of the patients was 67.56 kg (range 48–88 kg, SD 13.08). The mean concentration of creatinine in serum was 1.08 mg/dl (range 0.8–1.7 mg/dl, SD 0.26). The mean C_{max} was 6.07 µg/ml (range 3.75–9.53 µg/ml, SD 1.83). The mean C_{AH} at the first hour following levofloxacin administration was 1.37 µg/ml (range 1.17–1.6 µg/ml, SD 0.22) and the mean C_S was 5.27 µg/ml (range 4.5–6.53 µg/ml, SD 1.1). The mean ratio (R=C_{AH}/C_S) was 0.26 (range 0.24–0.3, SD 0.02). The mean C_{AH} at 125–270 min after levofloxacin administration was 1.39 µg/ml (range 0.82–1.98 µg/ml, SD 0.33), the mean C_S was 5.11 µg/ml (range 2.72–8.15 µg/ml, SD 1.68) and the mean ratio was 0.3 (range 0.15–0.53, SD 0.11). All patients except for one had a C_{AH} of >1 µg/ml 1 h after levofloxacin administration. The concentrations of levofloxacin in AH and serum are shown in Tables 1 and 2. Ratios versus time values are detailed in Fig. 1. None of the patients developed infection after surgery.

Table 1 Mean concentrations of levofloxacin in aqueous humour and serum

Parameter	Mean (μg/ml) (range, SD)	Mean ratio (R=C_{AH}/C_S) (range, SD)
Mean C_{max}	6.07 (3.75–9.53, 1.83)	–
Mean C_{AH} (first hour after LVF)	1.37 (1.17–1.60, 0.22)	0.26 (0.24–0.30, 0.02)
Mean C_{AH} (125–270 min after LVF)	1.39 (0.82–1.98, 0.33)	0.30 (0.15–0.53, 0.11)

LVF levofloxacin, C_{max} maximum concentration in plasma, C_{AH} concentration in aqueous humour, C_S concentration in serum (serum and aqueous humour were obtained simultaneously)

Table 2 Patient characteristics and concentrations of levofloxacin in aqueous humour and serum samples

Patient	Sex	Age (y)	Weight (kg)	C_{max}	C_s	C_{AH}	Time (min)[a]
1	M	78	59	5.61	4.78	1.37	160.00
2	F	87	48	9.53	8.15	1.25	135.00
3	M	85	55	8.02	7.99	1.66	175.00
4	F	93	60	6.56	5.99	1.83	225.00
5	M	66	80	4.57	3.32	0.82	240.00
6	F	82	57	8.71	7.06	1.98	210.00
7	M	79	80	4.61	–	1.20	190.00
8	F	88	60	–	6.53	1.60	120.00
9	F	63	65	–	4.48	1.19	125.00
10	F	77	88	5.04	3.93	1.20	180.00
11	M	67	66	3.75	2.72	1.43	225.00
12	F	77	80	–	4.50	1.33	90.00
13	F	73	88	–	4.78	1.17	90.00
14	M	61	63	5.34	4.39	0.99	270.00
15	M	77	52	4.67	3.19	1.54	240.00
16	F	83	80	6.40	4.84	1.66	235.00

F female, *M* male,– sample not available

[a] Time after levofloxacin administration (when samples for C_s and C_{AH} were obtained)

Infectious agents generally gain access to the eye as a consequence of intraocular surgery (60%) or following a penetrating injury of the globe [10]. The etiologic agents of acute postoperative endophthalmitis are microorganisms of the eyelid skin and preocular tear film; therefore, coagulase-negative staphylococci are responsible for about 70% of post-cataract surgery infections, followed by *S. aureus* and, rarely, other gram-positive microorganisms (*Streptococcus* spp., *Enterococcus* spp. and *Propionibacterium acnes*). However, infections are also caused by *Haemophilus influenzae*, *Moraxella catarrhalis* and other gram-negative aerobic organisms. Post-traumatic endophthalmitis is usually polymicrobial (*Bacillus cereus* or staphylococci together with gram-negative rods). Many of these bacteria are susceptible to levofloxacin [2, 7].

The substantial penetration of levofloxacin into AH found in our study is in agreement with the findings of other investigators [11] who analysed the penetration of levofloxacin (after oral administration of one 500 mg tablet or two doses given 12 h apart before surgery) into the aqueous and vitreous humour. Fiscella et al. [11] found that microbiologically significant levels of the antibiotic can be achieved rapidly in both the aqueous and vitreous humour after oral administration of two doses (group 1) but not after one single dose (group 2). Group 1 achieved mean

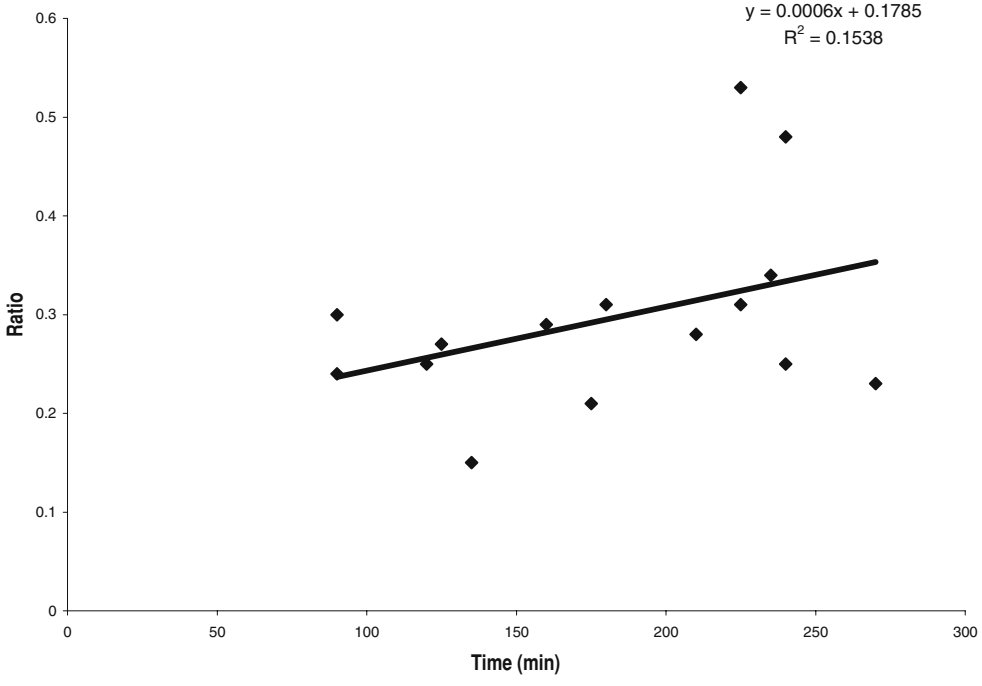

Fig. 1 Ratio of C_{AH} to C_S (R=C_{AH}/C_S) versus time for levofloxacin

aqueous and vitreous levels of 1.9 ± 0.97 μg/ml and 2.39 ± 0.7 μg/ml, respectively, but group 2 only achieved 0.59 ± 0.48 μg/ml and 0.32 ± 0.34 μg/ml, respectively.

The results of our study demonstrate that 1 h after the administration of levofloxacin, mean inhibitory aqueous MIC_{90} levels (≤2 μg/ml) were obtained against a majority of ocular pathogens, including methicillin-susceptible *S. aureus* strains and most gram-negative rods, but not *Pseudomonas aeruginosa*. Better penetration of ciprofloxacin into the vitreous of inflamed eyes was demonstrated in both the rabbit and the swine models [12]. Levofloxacin might also have similar pharmacokinetic-pharmacodynamic properties. The advantage of intravenously administering the antibiotic is that it can be performed in unconscious and severely ill patients. Moreover, this route of administration can more rapidly achieve a maximum concentration of levofloxacin in serum and in the eye; therefore, intravenous administration might be very useful when the antibiotic is used as prophylaxis before surgery and in the systemic treatment of endophthalmitis.

Nevertheless, systemic levofloxacin should not be relied upon as a sole antibiotic agent in the empirical treatment of suspected endophthalmitis, since for methicillin-resistant *S. aureus* and *S. epidermidis* the MIC of levofloxacin at which 90% of isolates are inhibited is >2 μg/ml (2 μg/ml in intermediate-level resistant and ≥4 μg/ml in resistant strains). These concentrations are higher than the mean levofloxacin levels obtained in AH (C_{AH} at the first hour following antibiotic administration: range 1.17–1.6 μg/ml and 0.82–1.98 μg/ml at 125–270 min after drug administration). However, the results from the present study indicate that levofloxacin may still be a useful adjunct for empirical treatment in postoperative or post-traumatic endophthalmitis in combination with another antibiotic with both good penetration into the human eye and activity against these resistant bacteria but not against gram-negative rods (i.e. linezolid) [13].

Early treatment of endophthalmitis is essential in order to avoid visual impairment; therefore, high levels of the chosen antibiotic in the eye early after administration are meaningful. The easy administration of levofloxacin (intravenous or oral) makes early treatment possible when a diagnosis is not yet confirmed, and direct injection of antibiotics into the eye might not be a first choice. The favourable pharmacokinetics of levofloxacin in the ocular AH provide support for trials that investigate the penetration of the antibiotic into the vitreous humour. This information could be helpful in determining the potential use of levofloxacin in the empirical cotreatment of endophthalmitis.

Acknowledgement The authors sincerely thank the nurses in the ophthalmology ward and surgery room without whose help this study would not have been possible.

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