Determination of Ciprofloxacin in Ocular Aqueous Humor by High Performance Liquid Chromatography: Comparison of Topical and Oral Administration

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Abstract

***Purpose*:** Fluoroquinolones are widely used antibiotics for prophylaxis of intra and postoperative infections in individuals undergoing cataract surgery. This study was designed to assess the penetration of ciprofloxacin into the ocular aqueous humor (topical only versus topical and oral administration).

***Methods*:** Studied population (n=47) consisted of two groups: group one (n=26) and group two (n=21). Group one received eye drop (one drop every six hours for three days before surgery and on the day of surgery topical medication was administered every 30 minutes with the last drop instillation maximum 4 hours before start of surgery). Group two received a combination of ciprofloxacin comprising of eye drop therapy as describe above plus oral dose (500 mg/twice a day starting three days before operation). Samples of aqueous humor were taken at the start of surgery. Ciprofloxcacin concentration was determined by high performance liquid chromatography (HPLC) with fluorescence detector.

***Results*:** Aqueous humor concentrations of ciprofloxacin in the patients who received combinations of eye drops and oral administration doses (mean 0.95 µg/ml) were significantly higher than patients receiving only eye drops (mean 0.23 µg/ml, P<0.001).

***Conclusion*:** The results for first group were below the minimum inhibitory concentration (MIC) values of Staphylococcus epidermidis, S. aureus, Streptococcus pneumoniae, Pseudomonas aeroginosa, Escherichia coli and Haemophilus influenzae. These results for the second group were over the MIC values of S. epidermidis, S. aureus, Streptococcus pneumoniae and Escherichia coli and below the MIC values of Pseudomonas aeroginosa and Haemophilus influenzae. These results demonstrate that topical ciprofloxacin can penetrate into the aqueous humor but it alone dose not seems to be prophylactically effective against most of the ocular pathogens. In most cases, combining the oral therapy with topical therapy increases the aqueous humor drug level and also is effective significantly against most of the ocular pathogens. This proposal is applicable for drug monitoring in patients undergoing prophylactic antibiotic therapy prior to surgery.

***Keywords*:** Ciprofloxacin, Ocular, High Performance Liquid Chromatography, Topical, Oral

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Introduction

The fluoroquinolones are broad spectrum antibacterial agents with activity against many of the important ocular pathogens including Staphylococci, Neisseria gonorrhoea, Haemophilus influenza, Enterobacterianceae and Pseudomonas aeruginosa.[1] Ciprofloxacin's bactericidal action results from interference with the enzyme DNA gyrase, which is needed for the synthesis of bacterial DNA.[2] In ophthalmology, it is widely used for the treatment of conjunctivitis and corneal ulceration. Ciprofloxacin has been also suggested as a possible agent in the prevention and treatment of intraocular infections.[3] Topical fluroquinolones are widely available and ophthalmic preparation has been formulated in 0.3% solution. Because of their broad spectrum of activity, they have the potential to be useful as first line therapy in external eye infection and keratitis, they may be a good choice for prophylaxis against postsurgical endophthalmitis.[1] Thus selection of a prophylactic antibiotic is dependent upon the ability to penetrate the surgical site with a concentration effective against pathogens and to provide a high enough concentration of antibiotic in the aqueous humor. Previous work suggests that combining oral and topical administration may greatly increase the aqueous antibiotic concentration.[4] The observation that ciprofloxacin penetrates particularly well in the aqueous using combining oral and topical administration regimen is supported by the recent observation of Leeming, Diamond and Celebi and colleagues.[1,5,6] High performance liquid chromatography (HPLC) with fluorescence detection is the most common analytical method utilized to determine ciprofloxacin concentrations in human biological fluids.[7]

This study was designed to investigate the penetration of topical administration and combining oral and topical administration of ciprofloxacin into the aqueous after different modes of administration.

Methods

Chemicals and reagents

Ciprofloxacin hydrochloride was obtained from Glaxo group Ltd by Glaxo operation Ltd (Greenferd, England). Acetonitrile (gradient grade for liquid chromatography), di-potassium hydrogen phosphate 3-hydrate, Sodium hydroxide and tetraethylammonium bromide were purchased from Merck (Darmstadt, Germany). Orthophosphoric acid was purchased from BDH chemicals Ltd poole (England). Ultra pure water was obtained using an E-pure system (Purelab option ELGA, England).

Stock solution

Stock solution of ciprofloxacin hydrochloride was prepared in ultra pure water (500 µg/ml). Stock solution was protected from the light with aluminum foil and kept at 4°C until used. It is reported that the glass surface absorbs ciprofloxacin and facilitates the degradation of ciprofloxacin under light.[8]

Calibration standards

Appropriate dilutions of the stock solution (500 µg/ml) were prepared using ultra pure water to obtain concentrations equal to 5, 2, 1, 0.5, 0.3 and 0.1 µg/ml. All calibration solutions were freshly prepared at every working day. Regression analysis of the calibration data was then carried out.

Study design

This study was a randomized controlled clinical study. The study involved 47 patients. All patients had a visually significant cataract for which the patient had been elected to have cataract surgery. Exclusion criteria included ongoing ocular inflammation, suspected infection, topical antibiotic treatment during the previous 7 days, active eye disease needing treatment, renal disease, a history of allergy to quinolones, age less than 18 years, pregnancy, diabetes mellitus and any drug that would interfere with ciprofloxacin and hepatic disease. All patients were asked to read and sign the informed consent and were randomized to one of the following groups:

- Group one (n=26) received eye drops 0.3% (one drop every six hours for three days before surgery and on the day of surgery topical medication was administered every 30 minutes with the last drop instillation the maximum of 4 hours before the start of surgery.

- Group two (n=21) received a combination of ciprofloxacin comprising of eye drop therapy as describe above plus oral dose (500 mg/ twice a day starting three days before operation).

Sample preparations

Following standard preparation and draping of the eye, typical for cataract surgery, a tuberculin syringe, was used as the initial entry into the eye. A single aliquot of aqueous of 0.1 ml was aspirated. All samples were labeled, immediately placed on an ice container and stored at -20°C until all samples were collected. Once all the samples were collected, they were sent to the Center for Cellular and Molecular Research laboratory at the Urmia University of Medical Sciences for analysis. The samples were thawed, mixed for one minute and centrifuged for 10 minute at 3000 g and 20 µl of the clear supernatant injected into the column of HPLC analysis to assay by reversed-phase HPLC method as previously described with slight modifications.[9,10]

Apparatus and chromatographic conditions

Chromatographic analyses were carried out on a Cecil Adept system Binary Gradient liquid chromatography (Cecil, England) equipped with a two adept CE 4100 dual pistons pump and a Ultrafluor chrom Tech fluorescence Detector (Model LC 305, USA). Chromatographic separation was performed on HI–5, C18–100A (10 cm × 4.6 mm id.) reversed-phase column (Hichrom, England), linked to a HI-5, C18-10C5 guard cartridge system at a flow rate of 1 mL min^{-1}. Both columns consisted of particle sizes equivalent to 5 µm. Manual sample injections were carried out using a Rheodyne model 77,25I injector with a 20 µl loop.

The mobile phase consisted of a mixture of acetonitrile and aqueous solution (20:80). The aqueous solutions were prepared by dissolving potassium dihydrogenophosphate (0.020 M), phosphoric acid (0.006 M), and tetraethylammonium bromide (0.012 M) in water. The PH of the mobile phase was adjusted to 3.0 by the addition of 2 M NAOH. The HPLC system was operated isocratically. The eluate was continuously monitored using a fluorescence Detector (λ_{ex} 338 nm and λ_{em} 425 nm). Peak height measurements and calculations the chromatograms were all carried out by an Integration pack program (version 3.2 of the Power Stream software package and the chromatography system manager CE 4900). All the analyses were performed at ambient room temperature (25°C) and without internal standard by duplicate. Among HPLC methods of analysis for ciprofloxacin in biological fluids, nearly half of these methods did not use internal standards.[8]

Statistical analyses

The sample size calculation was based on a study conducted previously by the investigators.[1,3,4,5] A statistical test was used to test hypotheses about difference between the concentrations of ciprofloxacin in ocular aqueous given topically alone or in a combination of topical and oral administration. A T-test was used to determine whether the means of the two groups were significantly different.

Results

Analysis of ciprofloxacin

The standard curve for ciprofloxacin passed through the origin and was linear over the range 0–10 µg/ml. The calibration graph was obtained by preparing standard samples of the ciprofloxacin by duplicate, with increasing concentrations of each analyte. The corresponding regression equation was Y=29.165X -1008 with an r^2 0.99, where Y is the peak height of ciprofloxacin and X is the concentration of ciprofloxacin (µg/ml). A representative calibration curve is shown in Figure 1 and a representative chromatogram is shown in Figure 2. The peak of ciprofloxacin appeared as sharp and well resolved peak with retention times of 2.2 minutes.

Precision and accuracy of the assay

The intra-day coefficient of variation was evaluated in the range of 0.3-4 µg/ml three times on the same day. The coefficients of variation (precision is expressed as the coefficients of variation, SD/mean × 100, CV%) for the method ranged between 2 and 4.84%. The inter-day coefficient of variation was similarly evaluated over a period of three consecutive days. The coefficients of variation for the method ranged between 6.15 and 6.99%.

The accuracy of the method (bias %) ranges between 89.59% and 105.50% for intra-day analysis and 95.84% and 104.15% for inter-days analysis. The limit of determination for ciprofloxacin was 0.1 µg/ml, Table 1.

Analysis of ocular aqueous

A total of 47 patients were studied (range 49-82 years). Twenty-six patients per treatment (group one) and 21 patients per treatment (group two) were enrolled. Fourteen were females and twelve males in group one and thirteen were females and eight males in group two. Table 2 summaries the demographic parameters and the mean and standard deviation for the aqueous antibiotic concentration of each group. The statistical comparisons have been done to show the matching of sex and age between the two groups, but these parameters were not significantly different for the two groups (P>0.05). The mean aqueous levels achieved after the combined topical and systemic administration of ciprofloxacin (mean 0.9562 µg/ml) were significantly greater than those observed with topical administration of ciprofloxacin alone (mean 0.2364 µg/ml, P<0.001). The concentration of ciprofloxacin is illustrated in Figure 3.

A brief literature review was conducted to identify the bacterial species most important pathogens causing postoperative endophthalmitis and their sensitivity to the ciprofloxacin studied. Determination of minimum inhibitory concentration (MIC) values for three species of bacterial was carried out by colleagues in the Department of Microbiology, School of Medicine (personal communication unpublished data).

Table 1. Intra-day and Inter-day precision and accuracy data for the high performance liquid chromatography determination of ciprofloxacin concentration

Concentration µg/ml	Intra-day Precision CV %	Intra-day Accuracy %	Inter-day Precision CV %	Inter-day Accuracy %
0.3	2	105.50	6.17	104.15
1	4.84	102.61	6.15	95.84
4	2.49	89.59	6.99	97.47
Mean	3.11	99.24	6.44	99.15

Table 2. Demographic and mean aqueous antibiotic concentration parameters

	Cip (topical alone)	Cip (topical + oral)	P-value
	26	**21**	**-**
Concentration (µg/ml) Mean (SD)	0.23 (0.23)	0.95 (0.63)	0.001[a]
Age (SD)	64.5 (9.49)	64 (10.2)	0.88[b]
Sex (male%)	12 (46%)	8 (38%)	0.57[c]

Cip, Topical ciprofloxacin alone (group 1)
Cip, Topical ciprofloxacin + oral ciprofloxacin (group 2)
SD: Standard deviation
a, b have analyzed by T-test
c has analyzed by χ^2 test

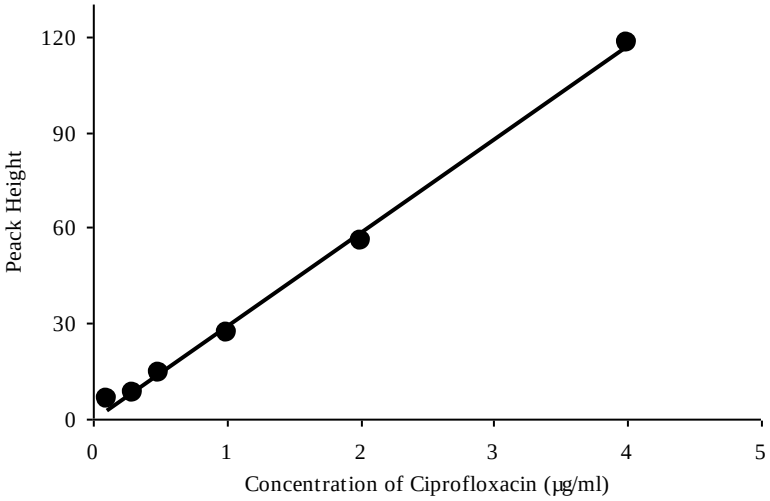

Figure 1. Representative calibration curve for the assay of ciprofloxacin concentration

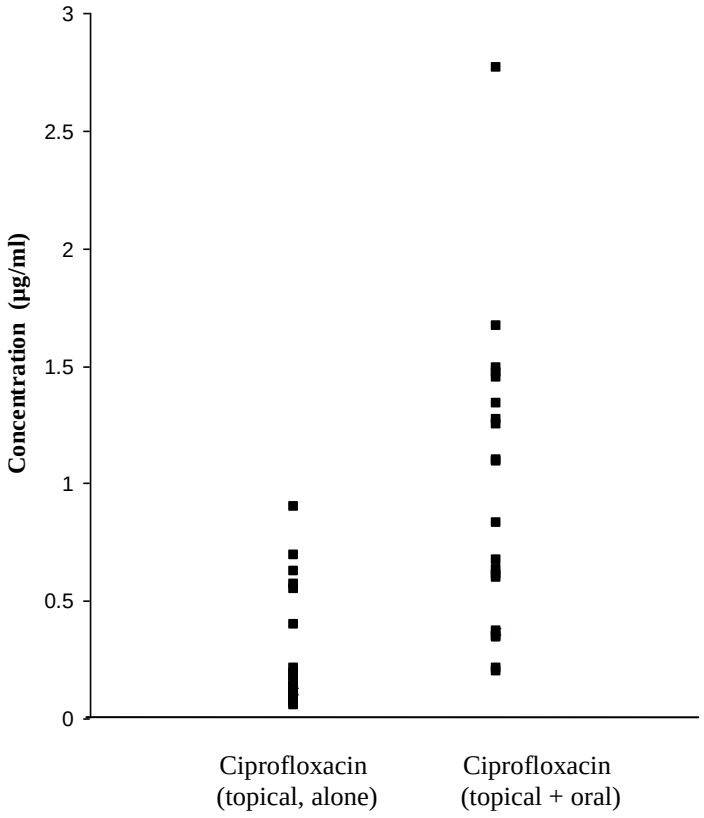

Figure 2. Ciprofloxacin concentration in the aqueous humour of patients who received administration of ciprofloxacin as a drop or ciprofloxacin as a drop and oral

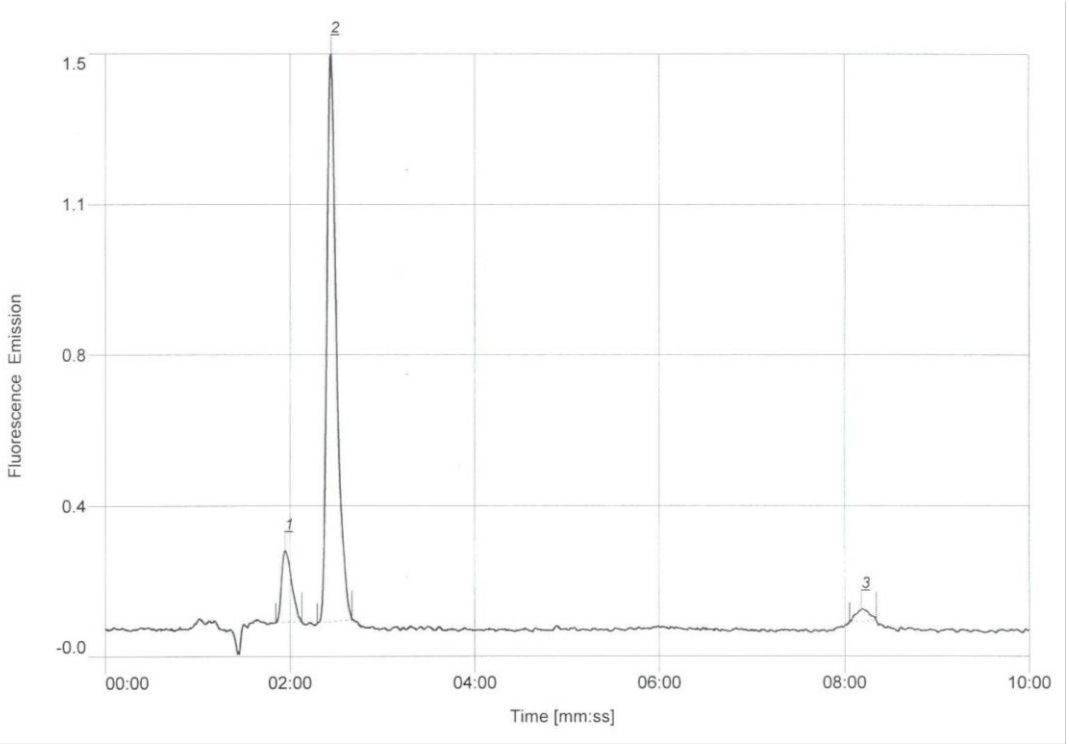

Figure 3. Representative high performance liquid chromatography chromatogram for the assay of ciprofloxacin, exciting at 338 nm and emission at 425 nm wavelength, retention time was 2.2 minutes, (2) Ciprofloxacin

Discussion

A literature review has been done to find systemic review or meta-analysis articles in this area using two electronic databases, PubMed Medline (1966-2011) and Cochrane Reviews Search (1993-2011), but we did not find any article in this field. Intraocular infection is one of the most serious complications of intraocular procedures or penetrating ocular trauma. Despite the use of the best available treatments, the visual prognosis of patients with intraocular infections remains guarded.[3] Ciprofloxacin is one of the drugs with strong fluorescence. Applying UV technique, fluorescence detection provides not only a higher sensitivity but also higher selectivity.[8] The analysis of ciprofloxacin in this study offers a simple, sensitive and accurate analytical method for the pharmacokinetic and pharmacodynamic study of ciprofloxacin, especially in ocular aqueous samples.

The results obtained for ciprofloxacin in this study confirm those previously published reports.[1,4,5] These results demonstrate that topical ciprofloxacin can penetrate poorly into the aqueous humor but it alone dose not seem to be prophylactically effective against most of the ocular pathogens. In most cases, combining the oral therapy with topical therapy increases the aqueous humor drug level. In the current study, the addition of oral ciprofloxacin to the topical regimen increased the ocular ciprofloxacin level by about 4 fold. Several previous studies of penetration of ciprofloxacin into rabbit and human eye have been reported.[1,3,4] There have been less reports concerning the penetration of ciprofloxacin and its action upon different germs. Leeming et al detected mean aqueous ciprofloxacin concentration of 0.22 µg/ml after 60 minutes instillation of the last drop based on application of 1houry single drop doses.[1] They have used 19 patients in this studies. Cantor et al have reported the concentration of ciprofloxacin in the aqueous 0.49 µg/ml for the topical regimen and 0.65 µg/ml for the combination of topical and oral regimen. In this study they have used 8 patients.[4] Ceki et al detected the mean aqueous ciprofloxacin concentration 0.23 µg/ml for the topical and

1.05 µg/ml for the topical plus oral regimens. They have used 12 patients in this investigation.[3] Since the dose and timing of the drug application have been different in all these above mentioned investigations from ours, a direct comparison would be unjust.

These results for the first group (eye drops) were below the MIC values of Staphylococcus epidermidis (0.25-0.58 µg/ml),[11,12] Staphylococcus aureus (0.25-1),[13-15] Streptococcus pneumoniae (1-1.5 µg/ml),[15-18] Pseudomonas aeroginosa (0.25-9.37 µg/ml),[19] Haemophilus influenzae (0.69-2 µg/ml)[20-22] and E.coli (0.14-1 µg/ml).[23]

The results for the second group were over the MIC values of Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli and close to the Streptococcus pneumoniae, and below the MIC value of Pseudomonas aeroginosa and Haemophilus influenzae (Table 3).

The most common Gram positive pathogens involved in postoperative endophthalmitis are staphylococcus epidermidis, staphylococcus aureus and Streptococcus pneumoniae species. The most common Gram negative organisms are Pseudomonas aeroginosa, Escherichia coli and Haemophilus influenza species.[24] In the present study, ocular aqueous humor ciprofloxacin levels following topical administration were below the therapeutic concentration required to inhibit those bacterias. In most of the subjects in the combined treatment groups, concentrations were above the MIC for Staphylococcus epidermidis, staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli, but below the MIC for some Gram negative organisms including Pseudomonas aeroginosa and Haemophilus influenza.

However, the use of the combined topical and systemic ciprofloxacin is more effective than the use of topical, ciprofloxacin in prophylaxis of endophthalmitis. These results suggest that combined therapy may have the potential to be effective for prophylaxis of endophthalmitis. However, even if the level of the antibiotic in the ocular aqueous was found to be higher than the MIC values that has been reported in the literature for a bacterium, can not be interpreted as a guarantee of successful therapy of ocular infection by members of that species, nor indeed does a ratio of below MIC value necessarily predict treatment failure.

Table 3. The most important pathogens causing postoperative endophthalmitis and the minimum inhibitory concentration values of these bacterial species, (µg/ml)

Organism	MIC value of ciprofloxacin	Ciprofloxacin concentration (topical alone)	Ciprofloxacin concentration (topical and oral)
Gram positive:			
Staphylococcus epidermidis, PTCC1112*	0.58*,0.25[10], 0.3[11] 0.25[20], 1[21]		
Staphylococcus aureus	0.5[23] 1[17,18,19],1.5[23]		
Streptococcus pneumoniae			
Gram negative:		0.2364	0.9562
Pseudomonas aeroginosa, ATCC27893*	9.37*, 0.63[12] 0.25[12] 0.14*, 1[22]		
Escherichia coli, ATCC25922*			
Haemophilus influenzae	2[14], 2[15] 0.69[16]		

*: Personal communication unpublished data
MIC: Minimum inhibitory concentration

Conclusion

The combination of topical and oral ciprofloxacin can be more effective in prevention of endophthalmitis in cataract surgery compared with topical therapy alone. However, it can be influenced by many factors such as the time of application of the drugs. Further investigations are required before this regimen or new generation of this drug to be recommended for this prophylactic aim.

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