CLINICAL STUDY

# Aqueous humour levels of topically applied ciprofloxacin and ofloxacin in the same subjects

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**Abstract**

***Purpose*** **To evaluate aqueous humour levels of topical 0.3% ciprofloxacin and 0.3% ofloxacin in the same subjects.**

***Methods*** **Thirty-two bilateral cataractous patients received topical 0.3% ciprofloxacin in one eye and 0.3% ofloxacin in the other eye before each cataract extraction. Eyedrops were repetitively instilled for 6 h. Aqueous humour samples were collected and assayed for drug concentrations by a method described originally by us using high-performance liquid chromatography.**

***Results*** **Mean aqueous ciprofloxacin and ofloxacin levels were 0.33 ± 0.04 μg/ml (mean ± SEM) and 1.34 ± 0.14 μg/ml respectively ($p<0.0001$).**

***Conclusion*** **Ofloxacin level in the aqueous humour is 4 times higher than that of ciprofloxacin in the same subjects.**

*Key words* Aqueous humour, Ciprofloxacin, High-performance liquid chromatography, Ofloxacin

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Fluoroquinolone antibiotics are active against a broad spectrum of Gram-negative and Gram-positive bacteria. They block DNA synthesis in bacteria.[1] Ciprofloxacin is used for the treatment of conjunctivitis and corneal ulceration.[2] The broad spectrum of activity, good penetration into the tissues and biological fluids and other favourable pharmacokinetic properties[3,4] increase the usefulness of ciprofloxacin in the treatment of ocular infections. Ofloxacin also offers several pharmacokinetic characteristics for better intraocular uptake over previous antibacterial agents.[5] Ofloxacin is used for the treatment of bacterial conjunctivitis, keratitis and ophthalmia neonatorum.[6–8]

The present study was designed to determine whether topical ciprofloxacin and ofloxacin reach concentrations in aqueous humour corresponding to the effective concentration range for certain common ocular pathogens and to compare their penetration and local bioavailability for the first time in the same subjects. We used a sensitive and reliable method described previously by us to measure ciprofloxacin[9] and ofloxacin[10] concentration in human ocular tissues.

## Materials and methods

Thirty-two bilateral cataractous patients (18 female, 14 male; mean age: 49.2 years) received topical ciprofloxacin in one eye and ofloxacin in the other eye before each cataract extraction. Surgery was performed at least 3 weeks apart in the same subjects. At the onset of surgery, an aqueous humour sample was obtained with a cannula through the anterior chamber from a limbal paracentesis tract. Collected samples were approximately 100–150 μl. Immediately after the samples were taken they were stored in a deep freeze at −70°C until the analysis. No patient had a history of ocular disease other than cataract or was receiving any antibiotic medication other than the solutions tested during the course of the study. Written informed consent was obtained from each patient and the study was approved by the ethics committee of the SSK Ankara Eye Hospital.

The same drug administration schedule was used for all patients. Starting 6 h before surgery two drops of solution were instilled in the eye to be operated on every 30 min for the first 3 h, followed by hourly instillation for the next 3 h. Drugs were administered by a nurse to ensure compliance. No adverse reactions were attributed to the antibiotic agents. All the samples were collected 30 min after the last dose.

All patients received 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride at 10 min intervals, beginning 2 h before surgery. Lids, eyelashes, skin surrounding the globe and conjunctiva were cleaned with 5% povidone-iodine immediately before the operation.



 

Ciprofloxacin and ofloxacin levels in aqueous humour were determined using a high-performance liquid chromatography (HPLC)–fluorescence detection method originally described by Basci *et al.*[9,10] HPLC grade methanol and acetonitrile were obtained from Baker (Phillipsburg, NJ) and analytical-grade citric acid and pipemidic acid from Sigma (St Louis, MO). The analytical column was a 100 mm × 8 mm internal diameter cartridge packed with 4 μm Novapak $C_{18}$ (Waters Chromatography Division, Milford, MA) compressed in a Radial-Pak cartridge holder (RCM 8 × 10, Waters) in conjunction with a pre-column module (Guard-Pak, Waters) containing a Novapak $C_{18}$ insert. All experiments were performed at a flow rate of 1 ml/min at ambient temperature. The mobile phase consisted of methanol–acetonitrile–0.4 M citric acid (3:1:10, v/v/v). The excitation and emission wavelengths were set to 278 nm and 450 nm respectively for ciprofloxacin, and 290 nm and 500 nm for ofloxacin. Drug concentrations were determined against a calibration curve constructed from their standard concentrations, and calculated from peak values and expressed as micrograms of drug per millilitre of aqueous humour.

The ciprofloxacin formulation contained a solution of 0.3% ciprofloxacin at pH 4.5 containing benzalkonium chloride (0.006%), mannitol (4.6%), and ethylene diamine tetraacetic acid (0.05%) with the addition of acetic acid and sodium acetate to a final osmolarity of 300 mosmol. The ofloxacin formulation used contained a solution of 0.3% ofloxacin at pH 6.4 containing benzalkonium chloride (0.005%) with NaCl added to an osmolarity of 300 mosmol.

Student's *t*-test was used to compare the data related to independent groups.

**Table 1.** *Aqueous humour levels of ciprofloxacin and ofloxacin in the patients (μg/ml)*

| Patient no. | Ciprofloxacin | Ofloxacin |
|---|---|---|
| 1 | 1.12 | 1.88 |
| 2 | 0.38 | 0.57 |
| 3 | 0.29 | 1.12 |
| 4 | 0.10 | 0.56 |
| 5 | 0.09 | 0.15 |
| 6 | 0.12 | 2.00 |
| 7 | 0.18 | 0.29 |
| 8 | 0.19 | 0.45 |
| 9 | 1.03 | 1.94 |
| 10 | 0.29 | 2.10 |
| 11 | 0.19 | 1.24 |
| 12 | 0.14 | 2.32 |
| 13 | 0.23 | 0.28 |
| 14 | 0.29 | 2.29 |
| 15 | 0.39 | 1.78 |
| 16 | 0.10 | 1.54 |
| 17 | 0.32 | 0.19 |
| 18 | 0.29 | 0.38 |
| 19 | 0.56 | 1.06 |
| 20 | 0.39 | 1.42 |
| 21 | 0.64 | 1.66 |
| 22 | 0.43 | 2.04 |
| 23 | 0.19 | 0.56 |
| 24 | 0.39 | 2.08 |
| 25 | 0.17 | 1.78 |
| 26 | 0.16 | 2.09 |
| 27 | 0.13 | 2.24 |
| 28 | 0.12 | 0.64 |
| 29 | 0.19 | 1.54 |
| 30 | 0.28 | 1.98 |
| 31 | 0.63 | 0.52 |
| 32 | 0.43 | 2.27 |
| Mean (SEM) | 0.33 ± 0.04 | 1.34 ± 0.14 |

## Results

Table 1 shows the aqueous humour levels of the drugs measured in each patient. Mean aqueous humour ciprofloxacin level was 0.33 ± 0.04 μg/ml (mean ± SEM) and that of the ofloxacin was 1.34 ± 0.14 μg/ml. Maximum and minimum concentrations produced by ciprofloxacin instillation in aqueous humour were 1.12 μg/ml and 0.09 μg/ml and those by ofloxacin instillation were 2.32 μg/ml and 0.15 μg/ml. There was a statistically significant difference between the mean aqueous humour levels of the drugs ($p<0.0001$).

## Discussion

Topical application of antibiotics has certain advantages. There is a reduced risk of side-effects and of resistance to applied antimicrobial drugs. Furthermore, compared with the systemic route of administration, the total amount of drug to which the patient is exposed is considerably lower. However, the cornea is a major barrier for the penetration of locally applied drugs into the anterior chamber. Drug penetration is limited by the physiological barrier of hydrophilic corneal stroma and lipophilic epithelium and endothelium. Only drugs with specific physicochemical properties are able to cross this barrier. Applied drugs have to be lipid and water soluble.[11] When drops are administered to the eye, only a small fraction of a single drop remains in the tear film. The remainder of the solution either flows almost immediately out of the eye or drains into the lacrimal canaliculi. Because of this high turnover of drug solution, topical drops must initially be applied frequently, i.e. every 15–30 min; this regime may then be replaced by less frequent applications.[12]

Ciprofloxacin is lipophilic enough to enter the eyeball and is preferred for the treatment of intraocular infections, along with a limited number of antibiotics with the same property.[13] When applied locally at a constant concentration into the conjunctival sac, ciprofloxacin penetrates into the aqueous humour and its concentration there is dependent on the number of doses instilled in human subjects without eye infection.[2,9,11,13–21] Topical ofloxacin also penetrates the cornea well and achieves therapeutic aqueous humour levels against a wide variety of bacteria.[10,18–24] Ciprofloxacin and floxacin have a similar spectrum of activity, with ciprofloxacin being moderately more active against Gram-negative bacteria, such as *Pseudomonas aeruginosa*, and ofloxacin moderately more active against Gram-positive bacteria, such as *Staphylococcus aureus* and

*Streptococcus pneumoniae*.[25] The major difference between ciprofloxacin and ofloxacin is their intrinsic solubility. Ofloxacin is the most soluble of all the fluoroquinolones in current medical use.[26]

In the present study, six doses of 2 drops instilled at 30 min intervals followed by three doses at 60 min intervals yielded a mean concentration in aqueous humour of 0.33 ± 0.04 μg/ml with 0.3% ciprofloxacin and of 1.34 ± 0.14 μg/ml with 0.3% ofloxacin. The difference between means was statistically significant. The local bioavailability of ofloxacin at the aqueous humour was 4 times better than that of ciprofloxacin in the same subjects, which is similar to the result we found in different subjects.[20] These data are also similar to those found by Donnenfield *et al*.[2] indicating ofloxacin levels in the aqueous humour were 4.5 times greater than those of ciprofloxacin in the different subjects.

We observed that a concentration in aqueous humour above the minimal inhibitory concentrations for 90% of strains tested ($MIC_{90}$) for *Staphylococcus epidermidis* (0.40 μg/ml) was achieved in 7 of 32 patients instilled with ciprofloxacin solution, and for *Staphylococcus aureus* (0.57 μg/ml) and *Pseudomonas aeruginosa* (0.50 μg/ml) in 5 and 4 of 32 patients respectively. The concentrations were below the therapeutic concentration required to inhibit *Streptococcus pneumoniae* (2 μg/ml).[13]

The mean aqueous humour concentration of ofloxacin was 1.34 ± 0.14 μg/ml. This value is greater than the $MIC_{90}$ of ofloxacin against most of the common ocular pathogens, including *Staphylococcus aureus* (0.40 μg/ml) and *Staphylococcus epidermidis* (0.80 μg/ml). Nine patients in the ofloxacin group reached the $MIC_{90}$ for *Streptococcus pneumoniae* (2 μg/ml). The concentrations were below the therapeutic concentration required to inhibit *Pseudomonas aeruginosa* (3.1 μg/ml).[27]

Our findings show that the aqueous humour penetration of topical ofloxacin is about 4 times higher than that of topical ciprofloxacin in the same subjects.

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