CLINICAL STUDY

Human aqueous and vitreous humour levels of ciprofloxacin following oral and topical administration

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Abstract

Purpose **To assess aqueous and vitreous humour ciprofloxacin concentrations following oral and topical administration of ciprofloxacin in patients with non-inflamed cornea and an intact crystalline lens, and to compare the concentrations of the drug given by either route.**
Methods **In this prospective study, 34 patients undergoing pars plana vitrectomy for various ocular pathologies were divided into two groups. Eighteen patients received 2 drops of 0.3% ophthalmic solution of ciprofloxacin every 30 min for 3 h and then every 60 min for the next 3 h, and 16 patients received a single oral dose of 1000 mg ciprofloxacin 6 h before surgery. The aqueous and vitreous humour samples were simultaneously harvested after oral or topical administration during pars plana vitrectomy to assess penetration of the drug. These samples were assayed for ciprofloxacin concentrations by a method described previously by us using high-performance liquid chromatography.**
Results **The aqueous and vitreous humour levels of ciprofloxacin were 0.59 ± 0.06 μg/ml (mean ± SEM) and 0.64 ± 0.06 μg/ml after oral and 0.44 ± 0.07 μg/ml and 0.22 ± 0.04 μg/ml after topical ciprofloxacin administration, respectively. Aqueous humour levels were not statistically significantly different following oral and topical administration ($p = 0.069$). However, the vitreous level of the drug after oral administration was significantly higher than that after topical administration ($p<0.001$).**
Conclusion **Ocular bioavailability of ciprofloxacin in aqueous humour following oral and topical administration is found to be similar when the drug was applied as described above. Penetration of ciprofloxacin into vitreous humour is less than that into aqueous humour after topical administration.**

Key words Aqueous, Ciprofloxacin, High-performance liquid chromatography, Oral, Topical, Vitreous

Ciprofloxacin is a synthetic fluoroquinolone antibacterial active against a broad spectrum of Gram-positive and Gram-negative ocular pathogens. It has proved to be a powerful topical antimicrobial for use as a single agent for treating keratitis and conjunctivitis.[1–3] Ciprofloxacin has also been suggested as a possible agent for prophylactic use in cases of endophthalmitis as it has demonstrated *in vitro* activity against *Staphylococcus* and *Bacillus* species and most Gram-negative organisms, including *Pseudomonas* species.[4,5] Like other quinolone anti-infective agents, ciprofloxacin blocks DNA synthesis in bacteria by inhibiting DNA gyrase.[6] Its broad spectrum of activity, low serum protein binding, adequate tissue penetration, favourable pharmacokinetics and relative safety in adults and in the aged following systemic administration provide evidence of the effectiveness of ciprofloxacin for the treatment of bacterial infections.[7–9]

This study was conducted to determine the aqueous and vitreous levels of orally and topically applied ciprofloxacin in patients with non-inflamed cornea and intact crystalline lens by using a sensitive and reliable method[10] described originally for measuring the ciprofloxacin concentration in human ocular humours by Basci *et al*.[10]

Materials and method

Collection of vitreous and aqueous humour samples from patients

Thirty-four patients undergoing pars plana vitrectomy for retinal detachment with proliferative vitreoretinopathy ($n = 25$), giant tear ($n = 6$) or penetrating eye injury with ($n = 2$) or without ($n = 1$) intraocular foreign body were selected for the study. Sixteen patients (mean age 51.1 ± 6.4 years; 9 men and 7 women) received a single oral dose of 1000 mg ciprofloxacin (Ciproxin; Bayer, Istanbul, Turkey) 6 h before surgery. Starting 6 h before the surgery, the other 18 patients (mean age 54.0 ± 6.8 years; 11 men and 7 women) received 2 drops of 0.3% ophthalmic solution of ciprofloxacin (Ciloxan; Alcon, Fort Worth, TX)

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into the conjunctival sac every 30 min for the first 3 h followed by hourly instillation for the next 3 h. Samples were collected 30 min after the last dose. Drug administration was done by a nurse to ensure compliance. No adverse reactions were attributed to the antibiotic agents.

Surgery was performed under retrobulbar anaesthesia. After perilimbal opening of the conjunctiva, a pars plana incision was made at 4 mm from limbus in the temporal inferior quadrant for the infusion line, and another incision was made in the temporal superior quadrant for the vitrectome. At the onset of vitrectomy, an aqueous humour sample was obtained with a tuberculin syringe from the anterior chamber through the limbus before the infusion line was opened. An undiluted vitreous sample from the mid-vitreous was collected into a syringe connected to the vitrectome. The volumes of the collected samples were 100–150 μl. Immediately after the samples were taken, they were stored in deep freezer at −70 °C. All the patients had a non-inflamed cornea, and an intact crystalline lens. Exclusion criteria were fresh vitreous haemorrhage, age less than 18 years, pregnancy, diabetes mellitus, allergy to quinolones, central nervous system, hepatic or renal diseases, and current treatment with theophylline, warfarin, non-steroidal anti-inflammatory drugs or cyclosporine. No antimicrobial medication other than ciprofloxacin was taken by any of the subjects during the course of the study. Written informed consent was obtained from each patient involved in the study. The study protocol was approved by the Ethics Committee of the SSK Ankara Eye Hospital, Ankara, Turkey.

Chemicals and reagents

High-performance liquid chromatography (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 ophthalmic solution of 0.3% ciprofloxacin used was preserved with benzalkonium chloride (0.006%) and contained as inactive ingredients sterile water, sodium acetate, acetic acid, mannitol (4.6%), EDTA disodium (0.05%), hydrochloric acid and sodium hydroxide (pH approximately 4.5).

Stock solutions of ciprofloxacin (1 mg/ml) and pipemidic acid (1 mg/ml) were prepared in HCl (0.01 N) and NaOH (0.2 M), respectively. Standard solutions of ciprofloxacin containing pipemidic acid (1 μg/ml) were prepared as internal standard by diluting the stock solutions of ciprofloxacin. All solutions were protected from light because of the light sensitivity of pipemidic acid.

Ciprofloxacin assays

Ciprofloxacin was measured by HPLC with fluorometric detection, as described by Basci *et al.*[10] The chromatography system consisted of a Varian 9100 autosampler, Varian 9002 solvent delivery system, Waters 470 scanning fluorescence detector and Waters 746 data module.

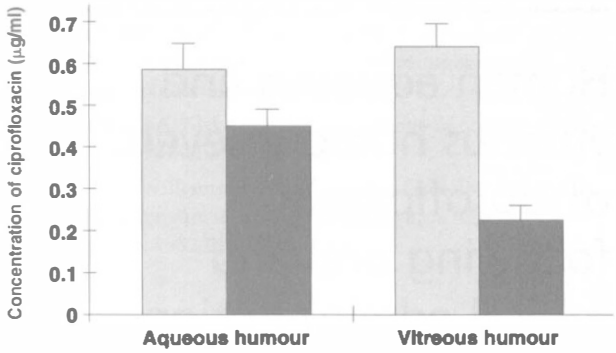

Fig. 1. *Comparison of aqueous and vitreous levels of ciprofloxacin obtained after topical (black columns) and oral (grey columns) treatments.*

The separation was performed on a Novapak C18 cartridge (100 × 8 mm i.d., particle size 4 μm, Waters) compressed in a Radial-Pak cartridge holder (RCM 8 × 10, Waters) in conjunction with a pre-column module (Guard-Pak, Waters) filled with Bondesil C18. The detector was set at 278 nm excitation wavelength with a 450 nm emission cut-off. Drug concentrations were determined against a calibration curve constructed from standard ciprofloxacin concentrations, and calculated from peak values and expressed as micrograms of ciprofloxacin per millilitre of vitreous and aqueous humour.

Statistical method

A Mann-Whitney *U*-test was used to compare the data related to independent groups.

Results

Levels of ciprofloxacin in aqueous and vitreous humours following topical and oral administration are shown in Fig. 1. Concentrations of ciprofloxacin in aqueous and vitreous humours following oral administration were 0.59 ± 0.06 μg/ml (mean ± SEM) and 0.64 ± 0.06 μg/ml, respectively (Table 1). The concentration range produced by oral ciprofloxacin in aqueous humour was 0.33–1.11 μg/ml. The range was 0.33–1.21 μg/ml in vitreous humour. The mean concentrations of ciprofloxacin after topical administration were 0.44 ± 0.07 μg/ml in aqueous and 0.22 ± 0.04 μg/ml in vitreous humour (Table 2). The concentration range of ciprofloxacin in aqueous humour was 0.10–1.12 μg/ml, and 0.07–0.57 μg/ml in vitreous humour. The ratio between the mean concentrations produced by oral and topical administration of ciprofloxacin was 1.3 in aqueous humour and 2.9 in vitreous humour.

The difference between the concentrations of ciprofloxacin in aqueous humour obtained with oral and topical administration was not statistically significant ($p = 0.069$). However, the concentration of ciprofloxacin

Table 1. *Intraocular ciprofloxacin concentrations (μg/ml) in the oral treatment group*[a]

Patient no.	Aqueous humour	Vitreous humour
1	0.33	0.88
2	0.35	0.70
3	0.72	0.33
4	0.35	0.71
5	1.02	0.53
6	0.47	0.46
7	0.48	0.41
8	0.39	0.51
9	0.68	0.70
10	0.49	0.56
11	0.71	0.58
12	0.87	0.71
13	1.11	0.94
14	0.41	0.53
15	0.42	0.47
16	0.59	1.21
Mean	0.59	0.64
SEM	0.06	0.06

[a]A single dose 1000 mg 6 h before surgery.

Table 2. *Intraocular ciprofloxacin concentrations (μg/ml) in the topical treatment group*[b]

Patient no.	Aqueous humour	Vitreous humour
1	0.22	0.19
2	1.09	0.31
3	0.16	0.33
4	0.15	0.09
5	0.56	0.08
6	0.16	0.16
7	0.30	0.08
8	0.11	0.53
9	0.41	0.09
10	0.57	0.22
11	0.56	0.19
12	0.38	0.18
13	0.39	0.29
14	0.70	0.31
15	0.38	0.16
16	1.12	0.57
17	0.49	0.12
18	0.10	0.07
Mean	0.44	0.22
SEM	0.07	0.04

[b]Two drops of 0.3% solution every 30 min for 3 h and then every 60 min for the next 3 h before surgery.

in vitreous humour produced by oral administration is significantly higher than that produced by topical administration ($p<0.001$).

No renal or hepatic function abnormality was encountered in the patients.

Discussion

Ciprofloxacin, *in vitro* the most active quinolone,[11] is lipophilic enough to penetrate into ocular humours and preferred for the treatment of intraocular infections, along with a limited number of antibiotics with the same property.[4,9] When applied locally at a constant concentration into the conjunctival sac, ciprofloxacin penetrates into the aqueous humour and its concentration is dependent on the number of doses instilled in human subjects without eye infection.[13–16] In the current study, six initial doses of 2 drops of 0.3% ciprofloxacin ophthalmic solution instilled at a 30 min interval followed by three doses at a 60 min interval yielded a mean concentration of 0.44 ± 0.07 μg/ml in the aqueous humour, which was comparable with concentrations produced by a single oral dose of 1000 mg ciprofloxacin. The data indicate that topical application of 0.3% ciprofloxacin solution has the potential to achieve microgram levels of drug in the aqueous humour depending on the dosing schedule.

Lesk *et al.*[4] found a mean aqueous ciprofloxacin level of 0.51 μg/ml following two oral doses of 750 mg ciprofloxacin 12 h apart, the latter of which was administered 2–12 h pre-operatively. In another study, Sweeney *et al.*[17] measured a mean aqueous ciprofloxacin concentration of 0.65 μg/ml following a single oral dose of 1000 mg ciprofloxacin given 4 h before surgery. The concentration in vitreous humour (0.59 ± 0.06 μg/ml) after oral ciprofloxacin administration as described in the present study is comparable to that in previous studies. We chose to sample 6 h after administration as we noted that this was the time when the highest vitreous levels were attained.

The penetration of ciprofloxacin into the vitreous humour was studied after systemic administration.[4,5,18–20] Additionally, we have published, for the first time, the level of penetration of ciprofloxacin into the vitreous humour following local instillation.[21] The current report is also the first comparative report of aqueous and vitreous concentrations of ciprofloxacin following oral and topical administration in human.

In a previous study, the mean vitreous ciprofloxacin level was found to be 0.49 μg/ml at 5 h 25 min after a single 750 mg oral dose.[18] In another study in which 750 mg oral ciprofloxacin was given at 12 h and repeated at 2 h before surgery, the range of intravitreal concentrations was 0.20–1.40 μg/ml with an average of 0.51 ± 0.35 μg/ml.[4] In patients receiving a dosage of 750 mg ciprofloxacin twice every 12 h starting 24 h before surgery, vitreal levels ranged from 0.40 to 0.90 μg/ml, with a mean of 0.56 μg/ml.[19] In the present study a similar vitreous ciprofloxacin level (0.64 ± 0.06 μg/ml) was attained following a single 1000 mg oral dose given 6 h before surgery. This dosing schedule seems to be the most convenient one for systemic treatment of intraocular infections.

In the current study, topical ciprofloxacin yielded a concentration in the vitreous humour of 0.22 ± 0.04 μg/ml after a total of nine doses scheduled as mentioned above. The results indicate that local administration of ciprofloxacin yields a vitreous humour concentration which is approximately one half the concentration in the aqueous humour. The reduced concentration in vitreous humour could be attributed to the additional barriers which the drug must penetrate to reach the vitreous space. There is also a larger volume of drug distribution

through the vitreous compared with the aqueous space. This difference in concentration between the two spaces disappeared after oral administration. Additionally, 1000 mg oral ciprofloxacin treatment 6 h before surgery can be used with a comparable effectiveness instead of repetitive topical application when needed to produce adequate aqueous and vitreous drug levels.

Cutarelli *et al*.[11] quote for ciprofloxacin a minimum inhibitory concentration inhibiting 90% of strains tested (MIC_{90}) at 1 μg/ml. Lesk *et al*.[4] quote as low as 0.4 μg/ml for *Staphylococcus epidermidis*, and up to 1 μg/ml for *Bacillus cereus*. Donnenfeld *et al*.[13] claim the most common organisms responsible for endophthalmitis to be *Staphylococcus epidermidis* (50%), *Staphyloccus aureus* (30%) and *Streptoccus* species (10%). For *Streptoccus* species the MIC_{90} may need to be 2 μg/ml, whilst the mean concentrations of antibiotic achieved in this research are well below 1 μg/ml. Effective treatment of bacterial endophthalmitis requires concentrations of at least twice the MIC_{90} for most bacteria,[4] and further studies are needed to establish the inhibitory quotient (concentration of antibiotic/MIC_{90}) required for effective prophylactic cover.

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