ELSEVIER

International Journal of Pharmaceutics 204 (2000) 97–100

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

The effects of prolonged acute use and inflammation on the ocular penetration of topical ciprofloxacin☆

Faruk Öztürk [a,*], Emin Kurt [b], Ümit Übeyt İnan [a], Selim Kortunay [c], Süleyman Sami İlker [b], Nursabah E. Başcı [c], Atila Bozkurt [c]

[a] *Department of Ophthalmology, Faculty of Medicine, Kocatepe University, Afyon, Turkey*
[b] *Department of Ophthalmology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey*
[c] *Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara, Turkey*

Received 22 February 2000; received in revised form 5 June 2000; accepted 8 June 2000

Abstract

Purpose: To study the aqueous and vitreous penetration of ciprofloxacin after prolonged acute topical administration and to investigate the effects of inflammation on drug penetration. *Methods:* A standardized model of intraocular infection after penetrating injury was made in the right eyes of eight rabbits. The intact left eyes were maintained as the control. Two drops of ciprofloxacin 0.3% eyedrops were instilled topically every 1 h for 7 h to all eyes of the rabbits. Aqueous and vitreous samples (100 μl) were obtained half an hour after the last drop. Instillation was continued for 7 h more and samples were obtained as before. Drug concentrations were measured using HPLC. *Results:* The mean aqueous humor levels of ciprofloxacin were: in control eyes 1.31 ± 0.78 μg/ml after 7 h and 1.85 ± 1.69 μg/ml after 14 h of instillation; in inflamed eyes 2.18 ± 1.02 μg/ml after 7 h and 2.91 ± 2.12 μg/ml after 14 h. The mean vitreous humor levels were: in control eyes 0.65 ± 0.44 μg/ml after 7 h and 0.72 ± 0.8 μg/ml after 14 h of instillation; in inflamed eyes 0.67 ± 0.77 μg/ml after 7 h and 1.01 ± 0.43 μg/ml after 14 h. However, the differences among the groups were not significant ($P > 0.05$). *Conclusions:* Ciprofloxacin penetration into aqueous humor was higher in 14-h topical application than that for 7 h. Inflammation increased the penetration of topical ciprofloxacin into aqueous while administered for 7 h and into both aqueous and vitreous humor while administered for 14 h.

Keywords: Ciprofloxacin; Penetration; Trauma; Endophthalmitis

1. Introduction

Endophthalmitis is a serious complication of ocular surgery and of penetrating ocular trauma. Although intravitreal injection of antibiotics is the primary route of antimicrobial management of endophthalmitis, the frequent and extended ad-

☆ Presented at the First Combined International Symposium on Ocular Immunology and Inflammation, Amsterdam, The Netherlands, June 27–July 1, 1998.

* Corresponding author. Present address: Dumlupınar Mh., Cevat Şakir Cd., Park Ap., 6/18 03200 Afyon, Turkey. Tel.: + 90-272-2131688; fax: + 90-272-2172029.
E-mail address: fozturk@aku.edu.tr (F. Öztürk).

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00483-X

ministration of antimicrobial eyedrops are usually included.

Ciprofloxacin is a fluoroquinolone antibiotic that has demonstrated in vitro activity against *Staphylococcus* and *Bacillus* species and most gram-negative organisms including *Pseudomonas* species (Neu, 1991). It has been suggested as a possible agent in the treatment and prevention of endophthalmitis (El Baba et al., 1992; Lesk et al., 1993).

There are several reports on the aqueous humor levels of ciprofloxacin after topical administration (O'Brain et al., 1988; Hobden et al., 1990; Donnenfeld et al., 1994; Leeming et al., 1994; Behrens-Baumann, 1996; Kalaycı et al., 1996), but there has been no study on the aqueous and vitreous penetration of ciprofloxacin after long-term topical administration and the effects of inflammation on ciprofloxacin penetration. The prolonged use and inflamed eye are more realistic models for studying intraocular penetration of topically administered antibiotics. It will be a potentially useful contribution if a topically applied antibiotic achieves a therapeutic concentration in the aqueous and vitreous humor in such conditions.

The present study has been designed to investigate the aqueous and vitreous humor penetration of topical ciprofloxacin after hourly administration for 7- and 14-h intervals in both inflamed and noninflamed rabbit eyes.

2. Materials and methods

Eight New Zealand albino rabbits of both sexes weighing between 2 and 3 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research. The rabbits were anesthetized with an intramuscular injection of a 50:50 mixture of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (30 mg/kg). To further reduce discomfort, the eyes were anesthetized using one to two drops of an oxybuprocaine (Benoxinate®). In the right eyes of all the rabbits, a standardized model of posterior penetrating ocular trauma was made and then repaired, similar to described by Cleary and Ryan (1979). Briefly, after a 360° peritomy, a # 11 blade was used to make a 5-mm laceration 2.5-mm posterior to the limbus. Prolapsing vitreous was excised and the wound was closed with interrupted 6-0 vicryl sutures, using microsurgical technique. Intraocular infection was induced by a midvitreous injection of 10^4 cfu/0.1 ml of American Type Culture Collection 25923 isolates of *Staphylococcus aureus* in the right eye of each rabbit under direct ophthalmoscopic control (Öztürk et al., 1999).

The animals were examined clinically at 4, 8, 12, 16 and 24 h after inoculation using biomicroscopy and indirect ophthalmoscopy. Inflammation of the vitreous cavity was assessed and graded by a masked observer (FÖ) according to a scale adopted from Peyman et al. (1975): 0, vitreous clear; 1, mild vitreal haze, good red reflex; 2, moderate vitreal haze, partial red reflex; and 3, total opacification of vitreous cavity, no red reflex. At each examination, animals were assessed three times, and an average of the three observations was used as the final grade. We ensured that all the animals inoculated developed grade 3 vitritis, characterised by obscuring of the posterior pole at 24 h.

Twenty-four hours after *S. aureus* inoculation, topical two drops of 0.3% ciprofloxacin (Ciloxan, Alcon) were administered to all eyes of the rabbits hourly, for 7 h. Aqueous and vitreous samples (100 μl) were obtained half an hour after the last drop. Instillation was continued for 7 h more and samples were obtained as before. The intact left eyes were maintained as the control. They were stored at $-20°$ until analysis. Drug concentrations were measured using high-pressure liquid chromatography analysis (Başcı et al., 1996).

Results are expressed as the mean $\pm$ S.D. Statistical analysis was done using the Wilcoxon rank sum test for comparison of 7- and 14-h values, Kruskal–Wallis one-way analysis of variance and Mann–Whitney *U*-tests for comparison between inflamed and control values. A *P*-value of < 0.05 was considered statistically significant.

3. Results

Mean concentrations of ciprofloxacin in aqueous and vitreous humor for the study groups

are listed in Table 1. Ciprofloxacin level in aqueous humor after 7-h instillation was higher than the 90% minimum inhibitory concentrations (MIC_{90}) of most common microorganisms responsible for endophthalmitis.

Aqueous and vitreous levels of ciprofloxacin in control eyes and inflamed eyes at 14 h were higher than those at 7 h. Mean aqueous levels of ciprofloxacin in inflamed eyes at 7 and 14 h and mean vitreous levels in inflamed eyes at 14 h were higher than those in control eyes. However, the differences among the groups were not significant ($P > 0.05$).

4. Discussion

To our knowledge, the penetration of ciprofloxacin after prolonged use for more than 6 h and the effects of intraocular inflammation on the penetration of topical ciprofloxacin into the eye have not been reported in the literature. In this study we found that the levels of ciprofloxacin in aqueous humor after 7-h instillation was higher than the MIC_{90} of most common microorganisms responsible for endophthalmitis (*S. epidermidis*, *S aureus*, *Pseudomonas species*, *B. cereus*) (Neu, 1991). Prolonged acute use (14 h) and inflammation increased the penetration of ciprofloxacin into the eye. However, this increase was not statistically significant. This may be due to the small size of the treatment group.

O'Brain et al. (1988) instilled six doses of ciprofloxacin 0.3% into intact rabbit eyes at 30-min intervals and found the drug concentration in the aqueous humor as 4.82 ± 2.15 μg/ml 30 min after the last dose administered. In another study, topical ciprofloxacin 0.75% was instilled to the rabbit eyes with an intact epithelium one drop every 15 min for 1 h followed by aqueous humor sampling 1 h later (Hobden et al., 1990). The aqueous humor level was found to be 4.2 ± 1.1 μg/ml. Leeming et al. (1994) instilled one drop of ciprofloxacin 0.3% into human eyes at 60-min intervals. In this study, drug concentration in the aqueous humor 10–60 min after the last dose administered was found to be 0.2 μg/ml. Kalaycı et al. (1996) reported that instillation of five doses of ciprofloxacin 0.3% into human eyes at 15-min intervals and nine doses of ciprofloxacin 0.3% into human eyes at 30-min intervals for 6 h yielded drug concentrations in the aqueous humor of 2.42 ± 1.42 μg/ml 30 min after the last dose administered. Donnenfeld et al. (1994) have found the penetration of topical ciprofloxacin as 0.072 μg/ml into human aqueous humor by applying two drops two times half hourly. According to these results, the penetration of ciprofloxacin seems to be proportional to the frequency and duration of application. Different drug administration regimens, variable time periods between drug application and aqueous humor sampling and different assay procedures may affect these results (O'Brain et al., 1988; Hobden et al., 1990; Donnenfeld et al., 1994; Leeming et al., 1994; Behrens-Baumann, 1996; Kalaycı et al., 1996). Furthermore, a species difference in the penetration of ciprofloxacin may exist.

Our study showed that topical instillation of ciprofloxacin is able to penetrate into vitreous humor, although in levels lower than the aqueous humor. Similarly, Behrens-Baumann and Martell (1988) reported the concentration of ciprofloxacin in the vitreous was near the detection limit following one dose of 1 mg subconjunctival injection. In our study, the mean vitreous drug concentrations exceeded the MIC_{90} of some ocular pathogens such as *S. epidermidis*, *S. aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* (Neu, 1991). The clinical importance of this penetration is unknown. The increased penetration of ciprofloxacin

Table 1
Ciprofloxacin levels (μg/ml) in aqueous and vitreous humor after topical instillation hourly for 7 and 14 h into the control and inflamed rabbit eyes[a]

	Aqueous humor		Vitreous humor	
	7 h	14 h	7 h	14 h
Control	1.31 ± 0.78	1.85 ± 1.69	0.65 ± 0.44	0.72 ± 0.80
Inflamed	2.18 ± 1.02	2.91 ± 2.12	0.67 ± 0.77	1.01 ± 0.43

[a] Concentrations of ciprofloxacin are given in μg/ml, mean ± S.D., $n = 8$ for each control and inflamed group.

into the eye in long-term topical instillation and in inflammation may be due to an increase in penetration via corneal and noncorneal routes (Doane et al., 1978; Ahmed and Patton, 1985; Ueno et al., 1994; Olsen et al., 1998).

Our results showed that topical ciprofloxacin 0.3% with prolonged use achieved aqueous humor levels above the MIC_{90} for common ocular pathogens. Higher aqueous concentrations of ciprofloxacin were obtained with prolonged use and in the presence of inflammation and also with higher vitreous concentrations.

References

Ahmed, I., Patton, T.P., 1985. Importance of the noncorneal absorption route in topical ophthalmic drug delivery. Invest. Ophthalmol. Vis. Sci. 26, 584–587.

Başcı, N.E., Bozkurt, A., Kalaycı, D., Kayaalp, S.O., 1996. Rapid liquid chromatographic assay of ciprofloxacin in human aqueous humor. J. Pharm. Biomed. Anal. 14, 353–356.

Behrens-Baumann, W., 1996. Absorption of topically administered ciprofloxacin, ofloxacin and gentamicin in the inflamed rabbit eye. Ophthalmologica 210, 119–122.

Behrens-Baumann, W., Martell, J., 1988. Ciprofloxacin concentration in the rabbit aqueous humor and vitreous humor following intravenous and subconjunctival administration. Infection 16, 54–57.

Cleary, P.E., Ryan, S.J., 1979. Method of production and natural history of experimental posterior penetrating eye injury in the rhesus monkey. Am. J. Ophthalmol. 88, 212–220.

Doane, M.G., Jensen, A.D., Dohlman, C.H., 1978. Penetration routes of topically applied eye medications. Am. J. Ophthalmol. 85, 383–386.

Donnenfeld, E.D., Snyder, A., Perry, H.D., Aulicino, T., Gombert, M.E., Snyder, R., 1994. Penetration of topically applied ciprofloxacin, norfloxacin and ofloxacin into the aqueous humor. Ophthalmology 101, 902–905.

El Baba, F.Z., Trousdale, M.D., Gauderman, J., Wagner, D.G., Liggett, P.E., 1992. Intravitreal penetration of oral ciprofloxacin in humans. Ophthalmology 99, 483–486.

Hobden, J.A., Reidy, J.J., O'Callaghan, R.J., Insler, M.S., Hill, J.M., 1990. Ciprofloxacin iontophoresis for aminoglycoside-resistant pseudomonal keratitis. Invest. Ophthalmol. Vis. Sci. 31, 1940–1944.

Kalaycı, D., Başcı, N., Akpek, E., Hasıripi, H., Bozkurt, A., 1996. Penetration of topical 0.3% ciprofloxacin into human aqueous humor. Ophthalmic Surg. Lasers 27, 21–24.

Leeming, J.P., Diamond, J.P., Trigg, R., White, L., Hoh, H.B., Easty, D.L., 1994. Ocular penetration of topical ciprofloxacin and norfloxacin drops and their effect upon eyelid flora. Br. J. Ophthalmol. 78, 546–548.

Lesk, M.R., Ammann, H., Marcil, G., Vinet, B., Lamer, L., Sebag, M., 1993. The penetration of oral ciprofloxacin into the aqueous humor, vitreous and subretinal fluid of humans. Am. J. Ophthalmol. 115, 623–628.

Neu, H.C., 1991. Microbiologic aspects of fluoroquinolones. Am. J. Ophthalmol. 112, 15S–24S.

O'Brain T.P., Sawusch M.R., Dick J.D., Gottsch, 1988. Topical ciprofloxacin treatment of *Pseudomonas* keratitis in rabbits. Arch Ophthalmol, 106, 1444–1446.

Olsen, T.W., Aaberg, S.Y., Geroski, D.H., Edelhauser, H.F., 1998. Human sclera: Thickness and surface area. Am. J. Ophthalmol. 125, 237–241.

Öztürk, F., Kortunay, S., Kurt, E., İlker, S.S., İnan, Ü.Ü., Başcı, N.E., Bozkurt, A., Kayaalp, O., 1999. Effect of trauma and infection on ciprofloxacin levels in the vitreous cavity. Retina 19, 127–130.

Peyman, G.A., Pague, J.T., Meihels, H.I., Bennett, T.O., 1975. Postoperative endophthalmitis: A comparison of method for treatment and prophylaxis with gentamicin. Ophthalmic Surg. 6, 45–55.

Ueno, N., Refojo, M.F., Abelson, M.B., 1994. Pharmacokinetics. In: Albert, D.M., Jakobiec, F.A. (Eds.), Principles and Practice of Ophthalmology. Saunders, Philadelphia, PA, pp. 916–929.