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Penetration of topical and oral ofloxacin into the aqueous and vitreous humor of inflamed rabbit eyes☆,☆☆

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Abstract

Purpose: This study aimed to investigate the penetration of topical and oral ofloxacin into aqueous humor and vitreous humor in post-traumatic endophthalmitis model in rabbits. *Methods:* A standardized intraocular infection after penetrating injury was made in the right eyes of 16 rabbits. Intraocular infection was induced by intravitreal injection of a suspension of *Staphylococcus aureus*. The intact left eyes were maintained as controls. The animals were divided randomly into two groups. (1) In the topical group, two drops of ofloxacin 0.3% eyedrops were instilled to both eyes every 30 min for 4 h. (2) In the topical-oral group, two doses of 25 mg/kg of ofloxacin at 12-h intervals were given orally, then the protocol of the first group was applied. Aqueous and vitreous humor samples were taken 30 min after the last drop. Ofloxacin concentrations were measured by using HPLC. *Results:* Mean aqueous levels of ofloxacin in control eyes were: 3.25 ± 2.55 μg/ml in topical group, 4.58 ± 5.39 μg/ml in topical-oral group. Mean aqueous levels in inflamed eyes were: 5.21 ± 4.55 μg/ml in topical group, 10.34 ± 8.88 μg/ml in topical-oral group. Mean vitreous levels of ofloxacin in control eyes were: 0.17 ± 0.07 μg/ml in topical group, 1.30 ± 1.23 μg/ml in topical-oral group. Mean vitreous levels in inflamed eyes were: 0.35 ± 0.22 μg/ml in topical group, 3.48 ± 2.69 μg/ml in topical-oral group. There was no significant difference among the groups ($P > 0.05$), however. *Conclusions:* The result of this study suggests that oral supplementation of ofloxacin to topical instillation increased the ocular levels of ofloxacin in the post-traumatic endophthalmitis model. Mean drug concentrations in aqueous and vitreous humors were above the 90% minimum inhibitory concentrations (MIC_{90}) for most of the common microorganisms causing endophthalmitis in all eyes, except in the vitreous humors of the intact eyes instilled topically.

Keywords: Ocular penetration; Ofloxacin; Post-traumatic endophthalmitis

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1. Introduction

Endophthalmitis is one of the most serious complications of intraocular procedures and penetrating ocular trauma. Although intravitreal antibiotics are the most important component of therapy in eradicating endophthalmitis, intravenous broad-spectrum antibiotics are commonly given to patients with penetrating ocular trauma to prevent post-traumatic endophthalmitis and are be used also as an adjunct to intravitreal antibiotics (Monk and Campoli-Richards, 1987; Osato et al., 1989; Todd and Faulds, 1991; Davis, 1996). Recent studies have shown that penetrating eye trauma and inflammation affects the ocular pharmacokinetics of certain antibiotics given intravenously, resulting in higher concentrations in traumatized (Alfaro et al., 1996) and inflamed eyes (Martin et al., 1990; Mounier et al., 1992; Meredith et al., 1995).

Ofloxacin is a fluorinated 4-quinolone antibiotic and is active against a wide spectrum of gram-positive and gram-negative organisms including various species of Staphylococcus, Streptococcus, and Pseudomonas (Auckenthaler et al., 1986; Osato et al., 1989; Todd and Faulds, 1991). It is among the fluoroquinolones considered promising for the treatment of ocular infections and shows nearly 100% bioavailability after oral administration (Monk and Campoli-Richards, 1987; Todd and Faulds, 1991). It was reported that ofloxacin penetrates the corneal and the blood-aqueous barriers and can achieve therapeutic aqueous humor levels above the minimum inhibitory concentration for many bacteria cultured in endophthalmitis in both topical and systemic administrations (Von Gunten et al., 1994; Donnenfeld et al., 1997). But higher concentrations of the antibiotic are required to inhibit *Streptococcus pneumonia* and *Pseudomonas aeruginosa* (Auckenthaler et al., 1986; Osato et al., 1989; Todd and Faulds, 1991).

Although there are several studies related to the penetration of ofloxacin into aqueous humor after topical or systemic administration (Yokota et al., 1986; Giamarellou et al., 1993; Von Gunten et al., 1994; Verbraeken et al., 1996; Başcı et al., 1997; Donnenfeld et al., 1997; Fiscella et al., 1997; Çekiç et al., 1998; Öztürk et al., 1999a), reports on penetration of systemic ofloxacin into aqueous humor and vitreous humor in inflamed eyes are limited (Mounier et al., 1992; Gatti and Panozzo, 1995; Öztürk et al., 1999a). Furthermore there are no data on penetration of topical or topical and systemic administration of ofloxacin in inflamed eyes. The purpose of the present study was to determine the aqueous and vitreous humors ofloxacin concentrations following topical instillation and oral medication in normal and inflamed eyes, and to compare the intraocular ofloxacin concentrations provided by two routes together and topical route alone.

2. Materials and methods

A total of 16 mixed-gender New Zealand white rabbits weighing between 2 and 3 kg were used in accordance with the ARVO Resolution on the Use of Animals in Research. The rabbits were anaesthetised with 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 anaesthetised using one to two drops of a oxybuprocaine (Benoxinate®, Thilo). A standardized posterior penetrating ocular trauma was made and then repaired, similar to that described by Cleary and Ryan (Cleary and Ryan, 1979), in the right eyes of all the rabbits. Briefly, after a 360° peritomy, a 5-mm incision 2.5 mm posterior to the limbus was made with a # 11 blade. Vitreous was excised and the wound closed with interrupted 6-0 vicryl sutures, using microsurgical technique. Intraocular infection was induced in the right eye of each rabbit under direct ophthalmoscopic control by a midvitreous injection of 10^4 colony forming units/0.1 ml of American Type Culture Collection 25923 isolates of *S. aureus*.

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 (F.Ö.) according to a scale adopted from Peyman et al. (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; an average of the three observations was used as the final grade. We ensured that all the inoculated animals developed grade 3 vitritis, characterized by obscuration of the posterior pole at 24 h.

Then 24 h after inoculation, the animals were divided equally and randomly into two groups, 'topical' and 'topical-oral'. The intact left eyes of the groups were maintained as controls. In the topical group, two drops of 0.3% ofloxacin (Exocin®, Allergan) were instilled in the eyes of the animals every 30 min for 4 h. In the topical-oral group, after overnight fast, two 25-mg/kg doses of oral ofloxacin were given as a crushed tablet in suspension by an intragastric tube at 12-h intervals. After the last oral dose, the protocol of the topical group was applied to the eyes of these animals. Then, 30 min after the last drops, aqueous humor and vitreous humor samples (100 μl) were taken. They were stored at −20°C until analysis. Ofloxacin levels in the aqueous and vitreous samples were measured by using HPLC (Başcı et al., 1997; Öztürk et al., 1999b).

Results are expressed as the mean ± S.D. Statistical analysis was performed by Kruskal–Wallis one-way analysis of variance for comparison among groups and then by Mann–Whitney *U*-test for comparison of two groups. A *P*-value of < 0.05 was considered statistically significant.

3. Results

The mean concentrations of ofloxacin in the aqueous and vitreous humor are listed in Table 1. The aqueous levels of ofloxacin were above the 90% minimum inhibitory concentrations (MIC_{90}) for most of the common microorganisms causing endophthalmitis in all eyes but the vitreous levels only in the inflamed eyes of the rabbits treated both topically and orally.

In the intact eyes, oral ofloxacin supplementation increased both the aqueous and vitreous levels of ofloxacin. In the inflamed eyes, drug levels were found to be higher than those achieved in the intact eyes. There was no significant difference among the groups, however ($P > 0.05$).

4. Discussion

Broad-spectrum, intravenous antibiotics are commonly suggested and administered as an adjunct to intravitreal antibiotics for treatment of endophthalmitis and for prophylaxis in penetrating ocular injuries (Martin et al., 1990; Meredith et al., 1995; Alfaro et al., 1996; Davis, 1996). The combination of systemic ofloxacin and topical ofloxacin was proposed as a single-drug therapy with broad-spectrum coverage for penetrating injury, surgical prophylaxis, and, possibly, the treatment of endophthalmitis (Fiscella et al., 1997). Inflamed eye is a quite realistic model for studying intraocular penetration of antibacterial agents and it would be potentially useful if the combination of systemic and topical ofloxacin achieved a therapeutic concentration in the aqueous and vitreous humor in inflamed eyes. The aim of this study was to evaluate whether oral supplementation of ofloxacin to topical instillation has any beneficial effect on the ocular ofloxacin penetration in the post-traumatic endophthalmitis model. The re-

Table 1
Mean aqueous humor and vitreous humor concentrations of ofloxacin after topical alone and combined topical and oral application in control eyes and inflamed eyes of rabbits[a]

Group	Aqueous humor		Vitreous humor	
	Topical	Topical-oral	Topical	Topical-oral
Control eyes	3.25 ± 2.55	4.58 ± 5.39	0.17 ± 0.07	1.30 ± 1.23
Inflamed eyes	5.21 ± 4.55	10.34 ± 8.88	0.35 ± 0.22	3.48 ± 2.69

[a] Concentrations of ofloxacin are given in μg/ml, mean ± S.D., $n = 8$ for each topical and topical-oral group.

sults showed that oral ofloxacin supplementation and post-traumatic inflammation increased both the aqueous and vitreous levels of ofloxacin, however the increase was not significant. This may be due to the small size of the treatment group.

The great variability observed in the mean ofloxacin levels may be related to mechanical and inflammatory insults. However, a statistically significant difference might be demonstrated in these eyes if there were sufficient numbers of cases. On the other hand, in the present study, ofloxacin was topically instilled after the oral administration. In clinical practice, it is likely that the topical drops and oral medication would be given concomitantly, which might actually increase the ocular ofloxacin level significantly to produce a statistically valid difference between the groups.

In control eyes, the mean aqueous humor level of ofloxacin (3.25 ± 2.55 μg/ml) was above the MIC_{90} in topical group for most of the common microorganisms causing endophthalmitis. The reported MIC_{90} of ofloxacin against ocular isolates is ~ 0.5 μg/ml for *S. aureus* (range, 0.125–4 μg/ml) and *Staphylococcus epidermidis* (range, 0.125–16 μg/ml), 2 μg/ml for *Streptococcus pneumonia* (range, 0.125–2 μg/ml), and 4 μg/ml (range, 0.25–8 μg/ml) for *Pseudomonas aeroginosa* (Osato et al., 1989).

The levels of aqueous ofloxacin humor in control eyes were higher than those reported in the literature (0.067–1.45 μg/ml) (Von Gunten et al., 1994; Donnenfeld et al., 1997; Başcı et al., 1997; Öztürk et al., 1999a), because these studies employed different antibiotic administration regimes, variable time periods between drug application and aqueous humor sampling, and different assay procedures. Therefore direct comparison is difficult. Donnenfeld et al. (Donnenfeld et al., 1997) used a topical drug application protocol which was similar to the present study. However, the aqueous ofloxacin concentrations obtained in this study were higher than those reported by Donnenfeld et al. (3.25 and 1.34 μg/ml, respectively) and in contrast, the vitreous concentrations were lower (0.17 and 0.37 μg/ml, respectively). One of the explanation of the greater ofloxacin penetration in the rabbit cornea may be the thickness of rabbit cornea which is 30% thinner than human cornea. The higher vitreous levels reported in the Donnenfeld et al. study may be due to the fact that all 20 patients had previously undergone cataract extraction and had a tear or absence of the posterior capsule (Donnenfeld et al., 1997). This factor allowed the ofloxacin to diffuse from the aqueous more readily into vitreous humor. Information on the penetration of topically applied ofloxacin into vitreous humor is limited to the Donnenfeld et al. study (Donnenfeld et al., 1997). In the present study, diffusion of ofloxacin into the vitreous humor after topical application was also observed (0.17 ± 0.07 μg/ml in control eyes and 0.35 ± 0.22 μg/ml in inflamed eyes). The penetration of ofloxacin into vitreous humor after topical application may be related to higher aqueous ofloxacin levels. Although physiologically the flow of aqueous humor is from posterior to anterior, diffusion of the drug may occur posteriorly based on concentration gradient.

The results obtained in rabbits showed that the addition of oral ofloxacin to the topical regimen increased the intraocular levels of ofloxacin. Although the size of increase in vitreous levels was lower than that obtained in the Donnenfeld et al. study (Donnenfeld et al., 1997), that observed in aqueous levels was similar. Because of species differences in the blood-retina barrier, diffusion of ofloxacin in rabbits may be more difficult than in humans. The effects of the addition of oral ofloxacin to topical therapy and inflammation were greater on the level of drug assayed in the aqueous humor. These results suggest that the blood-aqueous barrier may be more permeable than the blood-retina barrier in control and especially in inflamed eyes of rabbits. Inflammation may increase the drug absorption via corneal and/or non-corneal routes (Ahmed and Patton, 1985; Olsen et al., 1998).

In this animal model, oral ofloxacin supplementation to topical instillation and presence of inflammation increased the ocular concentration of ofloxacin. The statistically insignificance of this increase may be due to the small size of the treatment group. The mean aqueous and vitreous concentrations of ofloxacin were above the MIC_{90} for most of the common microorganisms causing endophthalmitis in all eyes, except in the vitreous humors of the intact eyes instilled topically.

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