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Effect of Inflammation on Intraocular Penetration of Intravenous Ofloxacin in Albino Rabbits

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The effect of inflammation on the intraocular penetration of ofloxacin was studied in 20 albino rabbits (New Zealand White). Inflammation was induced in the left eye by inoculation of a suspension of 10^9 CFU of heat-killed *Staphyloccus epidermidis* per 0.1 ml of saline solution (0.9%) in the midvitreous cavity. The other eye was kept as a control. Twenty-four hours following inoculation, ofloxacin was administered in the marginal ear vein at a dose of 15 mg/kg over 20 min with an infusion pump. Animals were sacrificed at different times up to 24 h following drug administration. Ofloxacin levels were determined in aqueous humor, vitreous humor, and serum by a bioassay. Inflammation was scored on the basis of perilimbal and corneal reactions and vitreoretinal statuses. Inflammation had a relevant effect on intraocular penetration of ofloxacin, with levels in the ocular fluids of the inflamed eye markedly exceeding the ones of the control eye. In the uninflamed eye, the levels were rapidly decaying below assay sensitivity and were no longer detectable at approximately 5 h following drug administration while they were still detectable in both ocular fluids of the inflamed eye at 24 h. Ofloxacin levels in the ocular fluids of the inflamed eye were superior to the MIC for several of the bacteria which commonly cause endophthalmitis, including *Staphylococcus epidermidis*, *Staphylococcus aureus*, most members of the family *Enterobacteriaceae*, *Haemophilus influenzae*, and strains of *Pseudomonas aeruginosa*.

Bacterial endophthalmitis is a severe intraocular infection complicating ophthalmic surgery and penetrative eye trauma. The incidence of endophthalmitis following cataract extraction has been reported to be in the range of 0.1 to 0.5% (5). The bacterial species isolated most frequently from ocular specimens are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus* spp., and gram-negative bacilli including *Pseudomonas aeruginosa*. However, virtually any bacterium may cause endophthalmitis (22). Current recommendations for the management of bacterial endophthalmitis include vitrectomy (11) with subsequent intravitreal injection of an empiric association of antibiotics allowing broad-spectrum coverage (e.g., cefazolin or vancomycin plus an aminoglycoside). Concomitantly with intravitreal injection, one or more subconjunctival injections and a course of systemic therapy are advisable (2, 13, 22). Following culture of vitreous humor and susceptibility testing of the isolate, treatment may be adjusted. Because of the toxicity associated with vancomycin and aminoglycosides, the usage of less-toxic agents for systemic therapy is preferred. The limit of cefazolin is represented instead by the increasing emergence of resistant staphylococci. Despite aggressive local and systemic therapy, visual prognosis of bacterial endophthalmitis remains poor and the need for treatments associated with a better outcome is strongly felt (22). Quinolones might have a role in the therapy of ocular infections (6), and several studies evaluating their intraocular penetration with various modalities of administration have been published (8, 9, 16, 23). Among the quinolones, ofloxacin is a well-tolerated drug with a spectrum of activity encompassing several of the bacterial species which are commonly isolated from patients with intraocular infections (20).

A major obstacle to the efficacy of systemic therapy is represented by the blood-eye barriers which hinder the penetration of drugs in the vitreoretinal complex. Thus, in order to select suitable antibiotics for adjunctive systemic therapy of endophthalmitis, the determination of the extent of penetration through the blood-eye barriers is fundamental. Two studies of the intraocular penetration of ofloxacin following the oral administration of a 200- (7) and a 400-mg (12) dose to patients undergoing cataract extraction showed that penetration in the aqueous humor is poor. However, these data have two limitations, common to all the studies of intraocular pharmacokinetics carried out in humans: (i) only the levels in the aqueous humor, which are often not predictive of the levels in the vitreous humor, were determined, and (ii) the effect of inflammation on intraocular penetration could not be evaluated. In course of inflammation of the ocular structures, the integrity of the blood-eye barriers may be altered and, consequently, intraocular pharmacokinetics may be modified. Recently, it has been reported that the concentrations of cefazolin are greater in the inflamed eye than in the control eye of albino rabbits (New Zealand White) (18). Thus, it appears necessary to characterize the intraocular penetration of antibiotics with animal models reproducing the physiopathologic conditions of the eye in the course of endophthalmitis.

Our study evaluates the effects of inflammation on ofloxacin kinetics in the vitreous and aqueous humors with an animal model of moderate panuveitis induced by the injection of heat-killed staphylococci.

Twenty albino rabbits (New Zealand White) (weight, 2.49 ± 0.13 kg [mean ± standard deviation]) were studied. All the procedures for induction of inflammation, ofloxacin administration, and ocular sample collection were performed with general anesthesia with intramuscular injection of ketamine hydrochloride (35 mg/kg of body weight) and xylazine hydrochloride (5 mg/kg). The study was approved by the Committee for Animal Studies at the Veterans Administration Medical Center, San Francisco, Calif.

Ocular inflammation was induced by a validated procedure

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(18). Briefly, an overnight culture of *S. epidermidis* ATCC 12228 in Mueller-Hinton broth (Difco, Detroit, Mich.) was centrifuged three times and resuspended in a 0.9% saline solution in order to eliminate the culture broth as an inflammatory stimulus. The final turbidity of the suspension was adjusted to equal the density of 4 McFarland standard (approximately 10^9 cells per ml). Staphylococci were subsequently heat killed by incubation of the suspension in a water bath at 80°C for 20 min. This procedure killed the bacteria, as confirmed by sterile cultures, but maintained intact the bacterial cell wall, as confirmed by microscopic observation. A volume of 0.1 ml of the bacterial suspension was injected through the pars plana in the midvitreous cavity of the left eye via a 30-gauge needle under direct visualization. The other eye was injected with 0.1 ml of 0.9% saline solution and used as a control. Paracentesis was not performed, since it is a well-recognized inflammatory stimulus. The above procedure has been shown (18) to induce a moderate panuveitis within 24 h following inoculation of the staphylococci suspension.

Ofloxacin was administered via an intravenous infusion in the marginal ear vein at a dose of 15 mg/kg (5 ml of aqueous solution at pH 3) over a 20-min period. Ofloxacin was given 24 h following the intravitreal inoculation of the bacterial suspension.

Animals were sacrificed at different times over a 24-h period following ofloxacin administration with 2 ml of sodium pentobarbital given intravenously. The aqueous humor was aspirated with a 23-gauge needle mounted on a tuberculine syringe. Eyes were enucleated and immediately frozen in a dry ice-acetone bath. Vitreous humor was subsequently obtained by dissection of the frozen eye (1) at the moment of ofloxacin level determination. A blood sample was collected by cardiac puncture. Blood was placed on ice for 20 min before centrifugation. Serum, aqueous humor, and frozen eyes were stored at −80°C until assayed for ofloxacin.

The degree of inflammation was scored as 1+, 2+, or 3+ on the basis of conjuctival and perilimbal reaction, corneal thickness, vitreous transparency, and retinal appearance.

Levels of ofloxacin in vitreous humor, aqueous humor, and serum were determined by a bioassay (4) with nutrient agar (Difco) as a growth medium and *Bacillus subtilis* ATCC 6633 (Difco) as a microorganism test. Each assay was performed in triplicate. A standard curve was prepared in rabbit serum at the following concentrations: 0.2, 0.4, 1, 3, and 10 μg/ml. The best prediction of standard concentrations was obtained by fitting the standard curve with the equation $y = a \cdot x^b$, where y is the predicted concentration, x is the diameter of the inhibition zone, and a and b are estimated parameters. The lower limit of quantification was 0.2 μg/ml. Assay variability throughout the study was 6.1, 5.4, 6.6, 5.2, and 3.6% at the concentrations of 0.2, 0.4, 1, 3, and 10 μg/ml, respectively. The deviation of the predicted concentrations of the standards from the corresponding nominal concentrations was less than 8%. The coefficient of determination (R^2) was always greater than 0.991. Preliminary experiments showed that diameters of inhibition zones in serum, aqueous humor, and vitreous humor were not significantly different (tested concentrations, 0.2, 1, and 10 μg/ml). Therefore, standards in serum were also used to determine ofloxacin levels in aqueous and vitreous humors. Ocular fluids were tested undiluted.

Ofloxacin levels in aqueous and vitreous humors in the inflamed eye were greater than the levels in the control eye in all the cases except for the concentrations in the vitreous humor of the rabbit studied at 1.25 h, which were identical for both eyes (Table 1).

The ratios of ofloxacin concentrations in ocular fluids to those in serum (Table 2) were greater than 1 from approximately 6 to 24 h in both aqueous and vitreous humor of the inflamed eye, while it was always very low for the fluids of the uninflamed eye.

Ofloxacin levels in both aqueous and vitreous humors of the uninflamed eye decayed rapidly and were detectable only up to

TABLE 1. Ofloxacin concentrations in serum and ocular fluids

Time (h)	Concn of ofloxacin (μg/ml) in[a]:				
	Serum	Aqueous fluid		Vitreous fluid	
		IE	UE	IE	UE
0.33	16.02	2.21	1.83	1.87	0.68
0.33	14.30	4.12	1.11	1.60	0.82
0.8	8.39	4.37	1.95	2.10	1.57
1.25	8.37	5.15	1.22	1.71	1.71
1.5	3.60	3.19	0.25	0.76	0.20
1.5	3.42	3.28	0.33	1.11	0.29
2.0	3.90	3.30	0.24	0.74	ND[b]
2.33	3.27	2.33	0.60	1.69	0.50
2.75	1.99	2.53	ND	1.30	1.15
3.25	1.41	2.77	0.20	1.08	0.30
5.0	1.47	0.49	ND	1.17	0.52
5.75	0.70	0.99	ND	1.34	ND
6.17	0.50	0.70	ND	1.28	ND
8.0	0.48	0.69	ND	0.75	ND
9.0	0.31	0.46	ND	0.58	ND
12.0	0.21	0.38	ND	0.77	ND
13.0	0.27	0.20	ND	0.87	ND
22.0	ND	0.26	ND	0.40	ND
24.0	ND	0.19	ND	0.31	ND
24.5	ND	0.23	ND	0.53	ND

[a] IE, inflamed eye; UE, uninflamed eye.
[b] ND, not detectable.

TABLE 2. Ratios of concentrations in ocular fluids to those in serum

Time (h)	Concn of ofloxacin in fluid of indicated type/concn of ofloxacin in serum[a]			
	Aqueous		Vitreous	
	IE	UE	IE	UE
0.33	0.14	0.11	0.12	0.04
0.33	0.29	0.08	0.11	0.06
0.8	0.52	0.23	0.25	0.19
1.25	0.62	0.15	0.20	0.20
1.5	0.89	0.07	0.21	0.06
1.5	0.96	0.10	0.32	0.08
2	0.85	0.06	0.19	—[b]
2.33	0.71	0.18	0.52	0.15
2.75	1.27	—	0.65	0.58
3.25	1.96	0.14	0.77	0.21
5	0.33	—	0.80	0.35
5.75	1.41	—	1.91	—
6.17	1.40	—	2.56	—
8	1.44	—	1.56	—
9	1.48	—	1.87	—
12	1.81	—	3.67	—
13	0.74	—	3.22	—
22	*[c]	*, —	*	*, —
24	*	*, —	*	*, —
24.5	*	*, —	*	*, —

[a] IE, inflamed eye; UE, uninflamed eye.
[b] —, ratio not determinable because levels were not detectable in ocular fluids.
[c] *, ratio not determinable because levels were not detectable in serum.

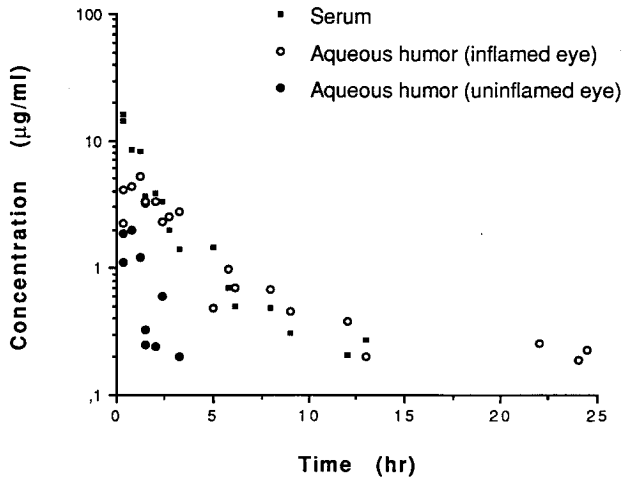

FIG. 1. Concentration-time profiles of ofloxacin in serum and aqueous humor.

about 5 h following drug administration, while in the inflamed eye they were still detectable at 24 h following administration. Figures 1 and 2 show the concentration-time profiles in aqueous humor and vitreous humor, respectively. In both figures, the levels in serum are reported for comparison with the concentrations in ocular fluids. No correlation was found between degrees of inflammation and drug levels in ocular fluids.

It is evident from this study that inflammation has a substantial effect on the intraocular penetration of systemically administered ofloxacin. The levels in the aqueous humor of the inflamed eye were higher than in the vitreous humor until 3 to 4 h following drug administration, while subsequently they were always higher in the vitreous humor. The observation of higher levels in the aqueous humor immediately following ofloxacin administration is consistent with the fact that ocular inflammation affects the tight intercellular junctions of the blood-aqueous humor barrier to a greater extent than it does the tight junctions of the blood-retina barrier (10). Thus, it seems reasonable that the increase of ofloxacin levels in the ocular fluids of the inflamed eye is caused by a greater penetration into the aqueous humor than into the uninflamed eye and by subsequent diffusion to the vitreous humor, while the extent of ofloxacin penetration from blood into the vitreous humor across the blood-retina barrier may be less affected by inflammation. Levels of ofloxacin in the vitreous and aqueous humors did not correlate with degrees of inflammation scored on the bases of corneal and retinal appearances as well as general conditions of the eye. This may indicate that even in the presence of mild-to-moderate inflammation the extent of leakiness of the ocular barriers is sufficient to enhance ofloxacin penetration into the eye.

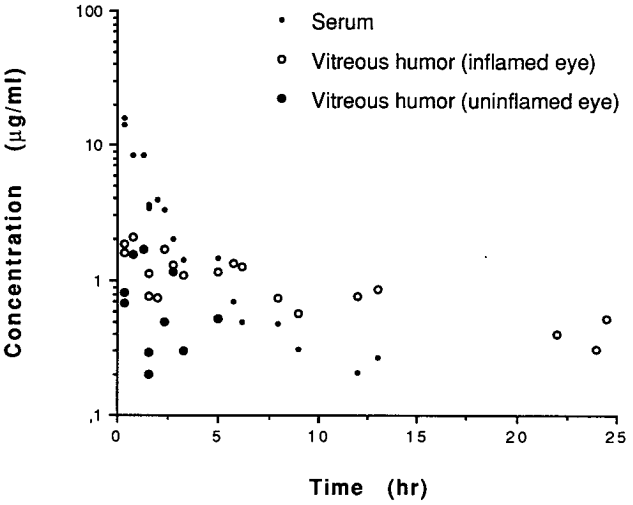

FIG. 2. Concentration-time profiles of ofloxacin in serum and vitreous humor.

It has been shown that a significant difference in the ocular pharmacokinetics of certain drugs may exist for albino versus pigmented rabbits (3, 8) because of drug binding to the pigmented tissues of the eye. We used albino rabbits for this study since the model of inflammation was validated with this rabbit phenotype. It has been reported that ofloxacin has a low affinity for melanin and that the antibacterial activity of ofloxacin combined with melanin is not significantly reduced (14). Whether the intraocular pharmacokinetics of ofloxacin is significantly different in pigmented rabbits should be evaluated.

Ofloxacin levels in the ocular fluids of the inflamed eye were higher than the MIC for numerous strains of *S. epidermidis*, *S. aureus*, *Haemophilus influenzae*, members of the family *Enterobacteriaceae*, and *P. aeruginosa* (19–21) for as long as 12 h following drug administration.

Extension of our findings to humans may be possible under a number of assumptions. It has to be pointed out that peak levels in serum were higher than the levels which can be reached with the maximum dose (400 mg) of intravenous ofloxacin tested in humans (15). Following the administration of a dose comparable with the ones tested in humans, concentrations in the ocular fluids of inflamed eyes would still be inhibitory for most of the bacteria isolated from patients with endophthalmitis, assuming no dose-dependent kinetics (17).

We administered ofloxacin intravenously in an attempt to avoid the introduction of oral bioavailability as a further element of difference between humans and rabbits. Even though an intravenous formulation has been recently introduced for ofloxacin, the oral route is still preferred since intravenous ofloxacin may be associated with severe skin reaction at the site of injection. However, because of complete bioavailability (17) the exposure to ofloxacin following an oral dose may be assumed to be comparable to that following an intravenous dose for patients without gastrointestinal problems.

In conclusion, we showed that inflammation triggers a significant increase of the intraocular levels of ofloxacin following systemic administration and that such levels may be sufficient to inhibit several of the bacteria which commonly cause endophthalmitis. We conclude that ofloxacin might be used in the treatment of endophthalmitis caused by susceptible bacteria. Studies of the intraocular pharmacokinetics of ofloxacin in aphakic and pigmented rabbits as well as efficacy studies in animal models of bacterial endophthalmitis are advisable in order to decide whether ofloxacin should be tested in humans.

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