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Efficacy and Ocular Penetration of Sparfloxacin in Experimental Streptococcal Endophthalmitis

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Gram-positive cocci are the most common pathogens in severe human eye infections. Streptococcal endophthalmitis is a devastating infection, and intravitreal antibiotic therapy is limited by retinal toxicity. Because few systemic antistreptococcal antibiotics penetrate into the vitreous, sparfloxacin, a newer quinolone with improved antistreptococcal activity, might be of interest. We therefore assessed its efficacy by the intravitreal route in a rabbit model of streptococcal endophthalmitis. The vitreal bacterial count (mean ± standard deviation $\log_{10}$ CFU per milliliter) was significantly reduced after an intravitreal injection of 800 μg of sparfloxacin (4.9 ± 0.7) relative to the counts in untreated control (7.1 ± 0.7) and pefloxacin-treated (7.8 ± 1.2) eyes. After systemic administration to rabbits, the maximum concentration of sparfloxacin in serum was 5.6 μg · ml^{-1} and the half-life was 7.5 h. Sparfloxacin exhibited very good penetration ratios in the vitreous (54%), cornea (76%), and lens (36%). In the vitreous, the levels of sparfloxacin remained greater than the MICs for most gram-positive cocci for up to 18 h. Further experimental studies are warranted to determine the efficacy of systemic sparfloxacin as adjuvant therapy in the treatment of human endophthalmitis.

The prognosis for patients with bacterial endophthalmitis remains extremely poor. Irreversible eye damage, especially to the retina, occurs during the first few hours of infection. The causative pathogens are varied: gram-positive cocci are the most frequent (11), but gram-negative bacilli can also induce fulminant endophthalmitis. The vitreous is a key compartment, since the infection can develop within it, but neither systemic nor topical antibiotics penetrate into it. Direct intravitreal injections are thus used, but they cannot be repeated because of their retinal toxicity. There is still a need for systemic antibiotics with broad antibacterial spectra of activity and good intraocular penetration.

New systemic quinolones like pefloxacin, ofloxacin, and ciprofloxacin have been shown to reach high levels in the aqueous and vitreous humors (4–6, 8, 15, 18, 20, 21), but their efficacies against streptococci are limited (7, 22, 23). Sparfloxacin and temafloxacin, even newer quinolones with enhanced antistreptococcal activity in vitro (1, 2, 7, 22), may thus be of interest in this setting.

In the present study, we used an experimental rabbit model of endophthalmitis to evaluate the antistreptococcal activities of sparfloxacin and pefloxacin following direct intravitreal injection into infected eyes. We then determined the full kinetics of sparfloxacin in the ocular humors and tissues when administered by the systemic route.

MATERIALS AND METHODS

Antistreptococcal efficacy. Eighteen healthy pigmented rabbits (Fauve de Bourgogne) were infected in the right eye by intravitreal inoculation with 10^6 CFU of *Streptococcus* sp. strain G, a strain for which the sparfloxacin MIC is 0.5 μg · ml^{-1}. The animals were anesthetized by intramuscular injection of ketamine (15 mg/kg of body weight) and by local instillations of oxyprocaine hydrochloride (0.4%). Prior to the injection, 0.1 ml of fluid was removed from the anterior chamber with a 25-gauge needle mounted on a tuberculin syringe to prevent an increase in intraocular pressure. The bacterial inoculum was injected by using a 30-gauge needle mounted on a tuberculin syringe. The needle was introduced 2 mm posterior to the limbus, near the superior rectus, and was directed toward the center of the vitreous. The antibiotic solutions were obtained by serial dilution of the preparation for systemic use in normal saline. The injection of 0.1 ml of antibiotic solution was performed slowly under direct visualization to avoid hitting the lens. At 20 h postinfection, six infected eyes received 800 μg of sparfloxacin (Rhône-Poulenc Rorer Laboratories, Paris, France) intravitreally, six eyes received 800 μg of pefloxacin (Roger-Bellon Laboratories, Neuilly-sur-Seine, France) intravitreally, and six eyes received normal saline intravitreally. The animals were killed with 2 ml of sodium pentobarbital given intravenously 24 h after antibiotic treatment. The whole vitreous was carefully dissected and homogenized with an Ultra-Turrax mixer. Serial dilutions of the vitreous were made in normal saline. A 100-μg sample was plated, and the plate was incubated for 18 h at 37°C. Bacterial counts are expressed as mean ± standard deviation $\log_{10}$ CFU per milliliter.

Pharmacokinetic studies. Fifteen healthy pigmented rabbits (Fauve de Bourgogne) received a single intramuscular injection of 50 mg of sparfloxacin per kg. Blood samples were obtained prior to sacrifice by intracardiac puncture. The animals were sacrificed at 1, 2, 4, 8, and 24 h (six eyes per time point) with 2 ml of sodium pentobarbital given intravenously. Aqueous and vitreous samples were aspirated via a 23-gauge needle mounted on a tuberculin syringe. The eyes were removed and cleansed of conjunctival tissue and blood to avoid contamination. They were immediately stored at −80°C to minimize antibiotic diffusion.

Assay. The frozen eyes were dissected to isolate the ocular tissues (cornea, iris, lens, chorioretina, and sclera); these were then homogenized with an Ultra-Turrax mixer. Sparfloxacin concentrations in ocular tissues, aqueous humor,

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TABLE 1. Ocular kinetics of sparfloxacin and temafloxacin in pigmented rabbits[a]

Tissue or fluid[b]	Mean ± SD AUC (μg · h/ml)		Tissue AUC/serum AUC (%)		$t_{1/2}$ (h)		C_{max} (μg/ml)		T_{max} (h)	
	Spa	Tema	Spa	Tema	Spa	Tema	Spa	Tema	Spa	Tema
Serum	37.5 ± 4.4	59.6 ± 4.1	100	100	7.5 ± 0.9	4.0 ± 0.3	5.6	11	1	2
Aq. humor	5.7 ± 1.4	6.5 ± 1.3	15	11	13.1 ± 2.6	2.5 ± 0.4	0.5	1.1	2	2
Vitreous	20.3 ± 7.0	3.5 ± 1.1	54	6	6.5 ± 0.7	2.3 ± 0.2	2.6	0.4	2	2
Cornea	28.3 ± 11.3	21.2 ± 9.6	76	35	12.4 ± 3.0	3.6 ± 0.4	3.3	1.5	1	2
Lens	13.4 ± 3.2	0.3 ± 0.2	36	0.5	∞[c]	∞	NA[d]	NA	NA	NA
Iris	1,688 ± 208.3	564.6 ± 14.5	4.5	945	∞	∞	98.4	31.8	2	8
Ch. retina	954.2 ± 266.1	682.2 ± 72.1	2.5	1,143	∞	∞	51.3	34.8	4	8
Sclera	106.5 ± 21.8	180.5 ± 91.9	284	303	∞	∞	5.3	9.6	4	8

[a] The mean AUC was determined through the course of the experiment (24 h). The half-life ($t_{1/2}$), C_{max}, and time to C_{max} (T_{max}) were determined from 1 to 24 h after a single intramuscular injection of 50 mg of sparfloxacin or temafloxacin per kg of body weight. Spa, sparfloxacin; Tema, temafloxacin.
[b] Aq. humor, aqueous humor; ch. retina, chorioretina.
[c] ∞, the half-life was too long for it to be evaluated during the course of the experiment (slope not different from 0).
[d] NA, not applicable; the constant could not be analyzed for the specific parameter indicated.

vitreous, and serum were assayed by high-performance liquid chromatography with UV detection at 280 nm.

The detection limit was 50 ng of sparfloxacin (the concentration of antibiotic that resulted in a signal-to-noise ratio of 4). The intraday coefficient of variation was 7% for the sparfloxacin high control (2,000 ng) and 10% for the sparfloxacin low control (50 ng).

Blood contamination in the vascularized tissues was evaluated by a hemoglobin microassay (sensitivity, 1 μg/mg), assuming that the hemoglobin concentration in blood is 13 g/100 ml and that the density of blood is approximately 1. Its contribution to the antibiotic concentration was considered negligible because of its very low-level presence (1.9, 3.5, and 0.6% in the iris, chorioretina, and sclera, respectively).

Pharmacokinetic analysis. Results were expressed per milliliter, assuming tissue densities of approximately 1. Areas under the concentration-time curves (AUCs) were calculated for the experimental period (0 to 24 h) by the trapezoidal rule method. Half-lives were calculated with the APIS software program (14) by using the maximum likehood estimation for a model with a monoexponential decrease. For tissue samples, a phase of increase of order 1 was used for the estimation. For some tissues, the half-life could not be evaluated because the slope did not differ from zero. The penetration ratio was defined as the tissue AUC/serum AUC.

Statistical evaluation. Comparisons of means of $\log_{10}$ CFU for treated eyes and control eyes were made by using Student's paired t test. P values of less than 0.05 were considered significant.

RESULTS

Antistreptococcal efficacy. Pefloxacin was ineffective. The bacterial count in the vitreous (7.8 ± 1.2 $\log_{10}$ CFU · ml^{-1}) was not significantly different from that in the controls (7.1 ± 0.7 $\log_{10}$ CFU · ml^{-1}).

In contrast, sparfloxacin led to a significant reduction in the bacterial count in the vitreous (4.9 ± 0.7 $\log_{10}$ CFU/ml) compared with that in the control ($P < 0.01$) and the pefloxacin-treated ($P < 0.01$) eyes.

Kinetics of sparfloxacin. The half-life of sparfloxacin in serum was 7.5 h, with a maximum concentration in serum (C_{max}) of 5.6 μg · ml^{-1} (Table 1). The most striking result was the high penetration ratio in the vitreous (54%); penetration was more moderate in the aqueous humor (15%) (Fig. 1). The C_{max} in the vitreous was 2.6 μg · ml^{-1}. The half-life in the vitreous (6.5 h) was similar to the half-life in serum (7.5 h), and the half-life in the aqueous humor was significantly longer (13.1 h). Systemic sparfloxacin maintained vitreal levels greater than the MICs for both staphylococci and streptococci for up to 18 h (Fig. 2). Sparfloxacin penetrated very well into the cornea and the lens (76 and 36%, respectively) and had a very long half-life. The C_{max} in the cornea was 3.3 μg · ml^{-1}. The levels of sparfloxacin were very high in the iris and chorioretina, and the half-lives were too long to be evaluated during the course of the experiment. These latter findings strongly suggest binding of the drugs to the pigmentary apparatus.

DISCUSSION

The first-line drugs used to treat bacterial endophthalmitis must have a broad spectrum of activity to cover the wide variety of causative agents. In addition, they must provide

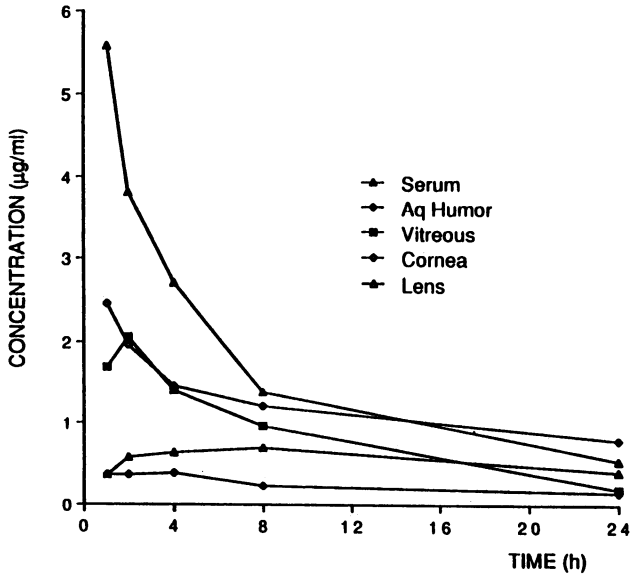

FIG. 1. Kinetics of sparfloxacin in serum, ocular tissues, and humors (Aq, aqueous) after a single intramuscular injection of 50 mg/kg in pigmented rabbits.

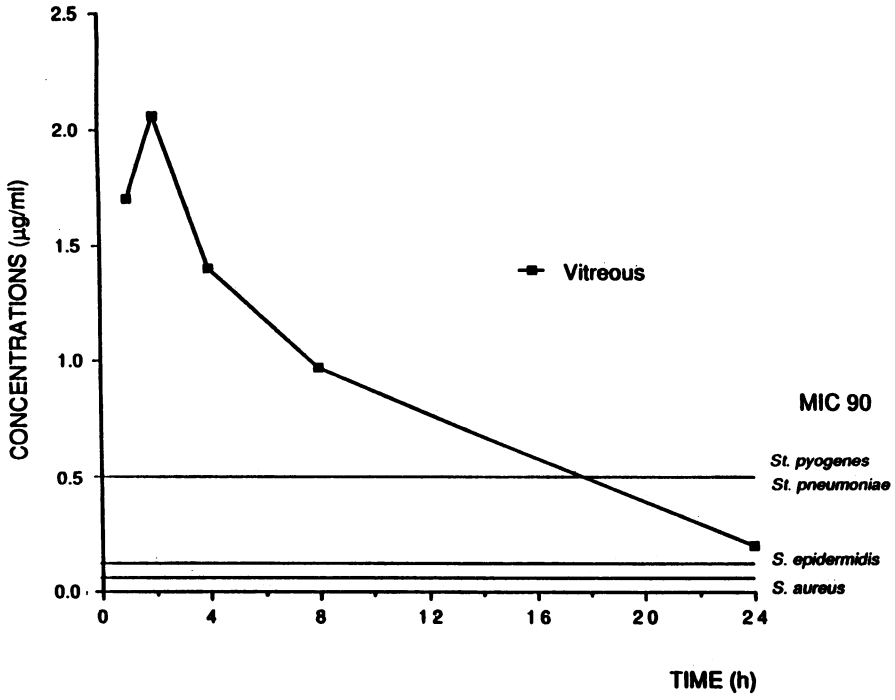

FIG. 2. MICs of sparfloxacin for 90% (MIC_{90}s) of the main pathogens found in humans with endophthalmitis compared with the vitreal levels of sparfloxacin after a single 50-mg/kg intramuscular injection in pigmented rabbits. St., *Streptococcus*; S., *Staphylococcus*.

effective concentrations in the vitreous and aqueous humors. Whatever the route of administration, antibiotics show poor intravitreal penetration because of ocular barriers: the corneal epithelium for the topical route and the blood-ocular (blood-aqueous and blood-retinal) barriers for the systemic route (10). The ability of drugs to cross these barriers depends especially on their molecular weights and lipophilicities.

By the systemic route, the ocular levels reached by conventional antibiotics are usually not sufficient to cure virulent endophthalmitis rapidly. Direct intravitreal injections of antibiotics provide transiently high vitreal levels, but the injections cannot be repeated because of toxicity. Antibiotics with good intraocular penetration by the systemic route are thus required as adjuvant therapy to maintain effective long-term levels in the eye.

New quinolones like pefloxacin, ofloxacin, and ciprofloxacin have greater intraocular penetration than conventional antibiotics (4–6, 8, 15, 18, 20, 21). Although they are active against a wide range of bacteria, their efficacies against streptococci are limited (7, 22, 23). In contrast, even more recent quinolones like sparfloxacin and temafloxacin have been shown to be effective in vitro against streptococci (1, 2, 23).

Our results demonstrate the antistreptococcal efficacy of sparfloxacin in vivo when administered directly into the infected rabbit vitreous; sparfloxacin significantly reduced the bacterial counts, whereas the same dose of pefloxacin did not. We obtained the same efficacy with sparfloxacin as we obtained with 800 μg of temafloxacin administered intravitreally (9) used under the same conditions; the bacterial count (4.6 ± 1.5 $\log_{10}$ CFU · ml^{-1}) was also significantly reduced relative to those in the control eyes ($P < 0.01$) and the eyes treated with pefloxacin ($P < 0.01$). The injected doses were high in terms of the MIC of these drugs for the test organism: 0.5 μg/ml for sparfloxacin, 1 μg/ml for temafloxacin, and 8 μg/ml for pefloxacin. The inefficacy of pefloxacin was surprising since the injected dose was several dilutions greater than the MIC. These results emphasize the diversity of factors involved in antibacterial activity in vivo and stress the importance of animal models in this setting.

For the kinetic study, we used pooled data from a population of rabbits to determine the complete distribution of the drugs in every eye structure. We could not thus obtain serial samples from individual rabbits, since this approach allows the determination of kinetics in the humors, but not in the tissues (16).

The half-life of sparfloxacin in serum is long in rabbits. In humans, the half-life of sparfloxacin administered orally is 18.2 h, permitting once-a-day dosing (17). Although the penetration of sparfloxacin into the aqueous humor was only moderate, its penetration into the vitreous was remarkably good. This discrepancy is surprising since the blood-aqueous barrier is known to be of the leaky type, in contrast to the blood-retinal barrier. The low levels and long half-life of sparfloxacin in the aqueous humor could be explained by an ability to penetrate into the neighboring cornea, lens, and iris, as attested to by the high penetration ratios in these tissues and the subsequent slow release from these three compartments into the aqueous humor. All these findings suggest a high cellular affinity of sparfloxacin. Indeed, sparfloxacin shows penetration into tissues, epithelial cells, and fibroblasts significantly greater than that of ofloxacin (19). It also enters easily into polymorphonuclear leukocytes (12), where it should retain its antibacterial activity. This might be related to its biophysical properties; its partition coefficient is several times greater than that of ofloxacin (12) and its molecular weight (392) is low.

Sparfloxacin might, like pefloxacin (8), gentamicin, and clindamycin (3), bind to the pigmentary apparatus. This

points to the need to use pigmented rabbits for the evaluation of antibiotic tolerability, kinetics, and efficacy.

Temafloxacin is a new quinolone that is similar to sparfloxacin but that has different kinetics. Compared with sparfloxacin, temafloxacin has a higher serum C_{max} and a shorter half-life (Table 1) when given under the same conditions (9). This difference is also found in humans, since the C_{max}s of sparfloxacin and temafloxacin after administration of 400 mg orally are 1.2 and 3.6 $\mu g \cdot ml^{-1}$, respectively, while the half-lives are 18.2 and 10.6 h, respectively (13, 17). Sparfloxacin shows better ocular penetration ratios than temafloxacin, especially in the vitreous (54 versus 6%), cornea (76 versus 35%), and lens (36 versus 0.5%).

Gram-positive cocci are the most common pathogens in human endophthalmitis (11). In rabbits, sparfloxacin given at 50 mg $\cdot$ kg^{-1} intramuscularly maintained vitreal levels superior to the MICs for both staphylococci and streptococci for up to 18 h. In humans, the validity of sparfloxacin administered orally at a conventional dose (400 mg) must be confirmed by kinetic studies of the aqueous and vitreous humors, since the serum C_{max} is lower (1.2 versus 3.6 $\mu g \cdot ml^{-1}$) but the serum half-life is longer (18.2 versus 7.3 h) in humans than in rabbits, resulting in similar AUCs. The main in vitro criterion used to predict antibacterial activity remains the inhibitory index, defined as the ratio between the absolute concentration of the antibiotic at the site of action and the MICs. However, this system shows limits, as attested to by the discrepancy between the antibacterial activities of sparfloxacin and pefloxacin (800 μg by intravitreal injection). Other factors can also interfere, stressing the need for experimental endophthalmitis models to determine the real in vivo activities of drugs.

In conclusion, when administered by the intravitreal route, sparfloxacin was effective against rabbit streptococcal endophthalmitis, while pefloxacin was not. By the systemic route, sparfloxacin showed good penetration into the eye, especially the vitreous. Further studies are required to assess the efficacy of sparfloxacin administered by the systemic route in the curative and/or preventive therapy of bacterial endophthalmitis. Indeed, systemic sparfloxacin might be of interest as adjuvant therapy for bacterial endophthalmitis in humans.

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