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Pharmacokinetics and ocular penetration of grepafloxacin in albino and pigmented rabbits

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The pharmacokinetics of grepafloxacin were determined in albino and pigmented rabbits following a single 10 mg/kg intravenous administration. The penetration of grepafloxacin into various ocular tissues was also determined after continuous intravenous infusion in both types of animal. Grepafloxacin showed a bicompartmental model of distribution in both pigmented and albino rabbits with significant differences in the pharmacokinetics between the two types of animal. After continuous intravenous infusion, significantly greater penetration of grepafloxacin was found in the iris, cornea and chorioretina of pigmented rabbits compared with albino rabbits.

Introduction

Fluoroquinolones are important therapeutic agents for the treatment of bacterial ocular infections,[1–3] with good bactericidal activity against many of the Gram-negative aerobes, as well as limited activity against the Gram-positive bacteria that cause these infections.[4–8] Moreover, they show excellent pharmacokinetic properties, such as long half-life, good tissue distribution, low protein binding, high antimicrobial activity and relatively few side-effects. The newer fluoroquinolones show good penetration into ocular tissues but with a spectrum of activity that includes coagulase-negative staphylococci.[9]

Fluoroquinolones have been found to be important therapeutic agents for the treatment of ocular infections, such as keratitis or conjunctivitis, when given by topical administration.[1,7,10] However, only a few authors have reported the systemic use of fluoroquinolones in ocular infections. Keren *et al.*[11] studied the ocular penetration of ciprofloxacin into both the vitreous and aqueous humour. Virgil-Alfaro *et al.*[12] studied the ocular penetration of ciprofloxacin in pigs and reported the treatment of experimental endophthalmitis in rabbits with various fluoroquinolones. Gatti & Panozzo[13] and Ng *et al.*,[14] using experimental models of endophthalmitis in rabbits, studied the ocular penetration of trovafloxacin and ofloxacin. Hatano *et al.*[15] compared the ocular penetration of lomefloxacin with that of other antimicrobial agents such as sulbenicillin and gentamicin, and finally, Cochereau-Massin *et al.*[1,7,8] and Liu *et al.*[16] compared the ocular penetration of various fluoroquinolones with that of imipenem, vancomycin and amikacin.

Grepafloxacin, although not currently marketed, exhibits good activity against many of the potential pathogens that may cause ocular infections.[10,17–20]

The aim of this study was therefore to evaluate the pharmacokinetics of grepafloxacin in albino and pigmented rabbits following intravenous administration, and establish whether there were differences between the pharmacokinetics or ocular penetration in these two types of animal.

Materials and methods

Animals were obtained and cared for in accordance with the recommendations of the European Community guidelines for the use of experimental animals.

Chemicals

Grepafloxacin was kindly supplied by Otsuka Pharmaceutical (Barcelona, Spain). Other chemicals were obtained from

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various commercial sources. All reagents were of HPLC grade.

Animal housing

Ten New Zealand rabbits (albino) and 10 Gigante de España rabbits (pigmented) weighing 3.5–4 kg were obtained from the Animal Experimentation Service of the University of Zaragoza. They were housed in rooms with a controlled temperature (20°C) and light cycle (12/12 h). The animals were maintained on water and standard laboratory chow *ad libitum* throughout the study. Each animal was judged to be clinically normal by physical examination before beginning the experiment. During this procedure, the heart and respiratory rates and the rectal temperature were also recorded.

Pharmacokinetic studies

Grepafloxacin was administered intravenously (iv) in the marginal ear vein of five pigmented rabbits and five albino rabbits as a bolus dose of 10 mg/kg body weight. Serial blood samples (1 mL) were drawn from a catheter inserted in the contralateral central ear artery at 0, 5, 10, 15, 30,45, 60, 90, 120, 180, 240 and 360 min into heparinized tubes. The samples were centrifuged and the plasma stored at –80°C until analysis.

Intraocular penetration studies

Grepafloxacin was administered in the marginal ear vein of five pigmented rabbits and five albino rabbits as an iv continuous infusion (4.6 mg/h) using a Mod M312 Gilson infusion pump (Pacisa, Zaragoza, Spain). Serial blood samples were drawn from a catheter inserted in the contralateral ear artery. Blood levels were measured until 200 min, in order to ensure that steady-state plasma concentrations of grepafloxacin (expected concentration 1.5 μg/mL) were achieved. The animals were then killed by iv administration of pentobarbital (30 mg/kg). Aqueous and vitreous humour samples were aspirated by puncture with a 23-gauge needle. The eyes were then removed and stored at –80°C. Frozen eyes were dissected and the ocular tissues, cornea, iris, lens, chorioretina and sclera, were obtained and stored at –80°C until analysis.

HPLC assay of grepafloxacin

Grepafloxacin was assayed in plasma and ocular tissues according to an HPLC method described previously.[21]

Pharmacokinetic analysis

The time course of the grepafloxacin plasma concentration (C) in both types of rabbit, and after using the Akaike criterion,[22] was described using the following equation:

$$C = C_1 \times e^{-\lambda_1 t} + C_z \times e^{-\lambda_z t} \quad \text{(Equation 1)}$$

where C_1 and λ_1 and C_z and λ_z are, respectively, the compartmental kinetic variables of distribution and elimination phases obtained by non-linear least squares regression analysis, using a weighting factor of $1/C^2$. The half-life in the distribution and elimination phases was calculated as $t_{1/2}\lambda_1 = \ln 2/\lambda_1$ and, $t_{1/2}\lambda_z = \ln 2/\lambda_z$ where λ_1 and λ_z are, respectively, the slope of the distribution and elimination phases.

Total body clearance was calculated as Cl_b = Dose/$AUC_{grepafloxacin}$, where $AUC_{grepafloxacin}$ is the area under the grepafloxacin blood concentration versus time curve, calculated by the trapezoidal rule to the last data point (C_{last}) and extrapolated to infinity by dividing C_{last} by the slope of the elimination phase. Finally, the volume of distribution of the central compartment (V_c) and the volume of distribution under the area (V_d) were calculated as V_c = Dose/C_z and V_d = Dose/($AUC_{grepafloxacin} \times \lambda_z$).

Statistical evaluation

The pharmacokinetic values obtained in the pigmented and albino rabbits are presented as mean ± S.D., except for the half-life, where the harmonic mean is shown. Comparisons between iv pharmacokinetic parameters from albino and pigmented rabbits were carried out using Student's unpaired t-test. The differences between the respective tissue concentrations were obtained by one-way analysis of variance (ANOVA). In all instances, $P < 0.05$ was considered to be statistically significant.

Results

The grepafloxacin plasma concentration–time plot following bolus injection for the two types of rabbit is shown in Figure 1, and the mean values for the grepafloxacin pharmacokinetic parameters are shown in Table 1. In all instances, the best fit for the pharmacokinetic data was obtained with a two-compartment model, as determined by the use of the Akaike criterion.[22] Following iv administration, grepafloxacin had a distribution phase in both albino and pigmented rabbits of ~40 min. When the pharmacokinetic parameters of both albino and pigmented rabbits were compared, significant differences were observed in C_1, C_z, λ_z, V_c, V_d, AUC and Cl_b ($P < 0.05$) (Table 1).

Figure 2 shows the mean plasma levels of grepafloxacin during continuous iv infusion of grepafloxacin in both albino and pigmented rabbits. After iv infusion of grepafloxacin, we found that the steady-state plasma concentrations of this drug were ~1.5 mg/L in both albino and pigmented rabbits.

In general, there were differences in the penetration of grepafloxacin into all ocular tissues, except aqueous humour, between albino and pigmented rabbits, with these differences

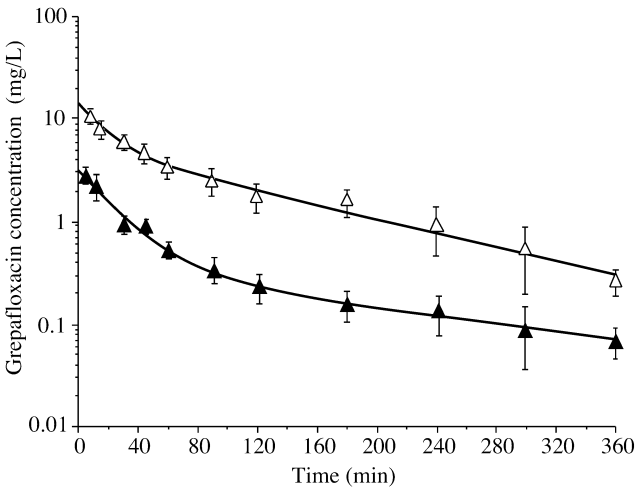

Figure 1. Mean ± S.D. plasma concentrations of grepafloxcin following an iv bolus dose of 10 mg/kg grepafloxacin. Filled triangles, pigmented rabbits; open triangles, albino rabbits.

reaching statistical significance for the chorioretina, cornea and iris (Table 2).

Discussion

Drugs used to treat eye infections must have a broad spectrum of activity and must reach effective concentrations in ocular tissue, especially in the vitreous and aqueous humours. However, when administered by the systemic route, many antibiotics show poor ocular penetration and the levels reached are often insufficient to cure virulent endophthalmitis.[1] Thus, new drugs that offer both good ocular penetration by the systemic route and a broad spectrum of activity against the potential pathogens in ocular infections are required.

Following iv administration of grepafloxacin the best pharmacokinetic fit of the plasma concentration was obtained for a two-compartment open model. To date there are no other reports on the pharmacokinetics of grepafloxacin in rabbits, with all the published data for grepafloxacin obtained from humans or in rats.[10,17–20,23–25] Our results are similar to the findings reported for the pharmacokinetics of other fluoroquinolones such as ciprofloxacin, sparfloxacin and enrofloxacin.[5,16,26–29] In our studies, the distribution kinetics of grepafloxacin were characterized by a large V_d, which agrees with findings reported in other animal species.[5] In our studies, when the pharmacokinetic parameters were compared between albino and pigmented rabbits, the latter exhibited higher values of V_d, Cl_b and $t_{1/2}$ than the former. Similar differences have also been described by other authors when the pharmacokinetics of pefloxacin was compared between albino and pigmented rabbits.[1]

In this article, we have also studied the penetration of grepafloxacin into ocular structures after continuous iv infusion of grepafloxacin, to steady-state plasma concentrations. We found clinically relevant concentrations of grepafloxacin in all ocular tissues, with the highest concentrations found in the iris and chorioretina of pigmented rabbits. Cochereau-Massin *et al.*[1] described a similar result working with pefloxacin, and other authors have demonstrated that penetration of sparfloxacin or moxifloxacin in the iris and ciliary body is related to the melanin concentration of these tissues.[30–32] A

Table 1. Pharmacokinetic parameters of grepafloxacin in albino and pigmented rabbits given a single iv administration of 10 mg/kg body weight

Parameter (units)	Albino (mean ± S.D.)	Pigmented (mean ± S.D.)
C_1[a] (mg/L)	13.9 ± 1.5	3.0 ± 0.8
λ_1 (min)	0.0737 ± 0.0162	0.0591 ± 0.0431
C_z[a] (mg/L)	4.3 ± 1.0	0.5 ± 0.2
λ_z[a] (min)	0.0098 ± 0.0018	0.0054 ± 0.0028
$t_{1/2}\lambda_1$ (min)	9[b]	11[b]
$t_{1/2}\lambda_z$ (min)	71[b]	126[b]
V_c[a] (mL/kg)	723.9 ± 82.0	3576.0 ± 969.2
V_d[a] (mL/kg)	2469.5 ± 305.5	2067.7 ± 11350.3
AUC[a] (μg/mL/min)	615.2 ± 151.1	139.44 ± 19.20
Cl_b[a] (mL/min.kg)	17.06 ± 4.16	72.74 ± 9.40

These terms are related to rate constants for the specific two-compartment pharmacokinetic model. C_1 and C_z are the coefficients and λ_1 and λ_z are the the exponents derived by non-linear least squares regression of concentration data to equation $C = C_1 \times e^{-\lambda_1 t} + C_z \times e^{-\lambda_z t}$. $t_{1/2}\lambda_1$ = distribution half-life; $t_{1/2}\lambda_z$, elimination half-life; V_c, volume of the central compartment; V_d, apparent volume of distribution calculated by the area method; AUC, area under the blood concentration time curve; Cl_b, body clearance.
[a]Significantly different.
[b]Harmonic mean.

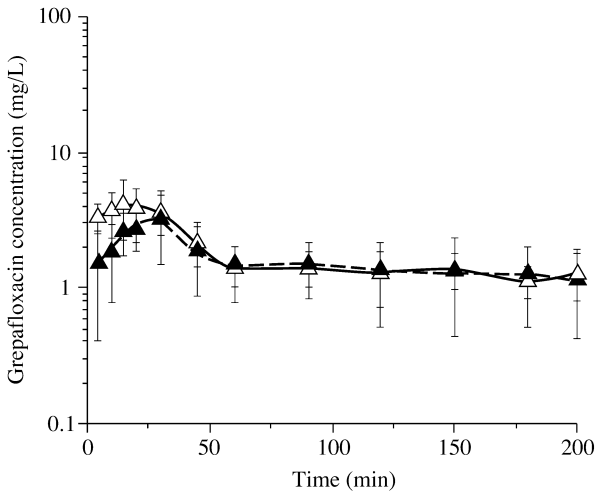

Figure 2. Mean ± S.D. plasma concentrations of grepafloxacin during continuous iv infusion in albino and pigmented rabbits. Filled triangles, pigmented rabbits; open triangles, albino rabbits.

similar influence of ocular pigments has been described for a number of other drugs, such as mydriatics, aminoglycosides or other fluoroquinolones.[33–35]

For all ocular structures, the penetration of grepafloxacin was found to be higher in pigmented than in albino rabbits. The degree of penetration was influenced by tissue vascularization, in that vascular tissues, such as the chorioretina and the iris, showed grepafloxacin ratios > 1, while non-vascular tissues, such as the aqueous humour, vitreous humour and lens, showed grepafloxacin ratios < 1 (Table 2). However, in spite of the fact that the sclera is a non-vascular tissue it showed ratios > 1. This tissue is very difficult to separate from the chorioretina and contamination from chorioretina could explain this concentration finding. This may also explain the findings of Cochereau-Massin *et al.*,[1] who found high concentrations in the sclera when studing the penetration of pefloxacin into ocular structures.

Table 2. Concentration and tissue to serum ratio of grepafloxacin following continuous iv infusion

	Albino		Pigmented	
Tissue	concentration (ng/g or ng/mL)	ratio	concentration (ng/g or ng/mL)	ratio
Plasma	1830	–	1314	–
Chorioretina	3367	1.84	40248	30.63[a]
Sclera	2580	1.41	4336	3.30
Iris	2361	1.29	53940	41.05[a]
Cornea	1116	0.61	2037	1.55[a]
Lens	293	0.16	526	0.40
Vitreous h.	366	0.20	762	0.58
Aqueous h.	458	0.25	276	0.21

Data are mean of five albino and five pigmented rabbits.
[a]Significantly different.

In conclusion, we have been able to demonstrate that grepafloxacin, a third-generation fluoroquinolone, has good capacity to cross the various ocular barriers after systemic administration, to achieve concentrations above those needed to inhibit bacteria that cause intraocular infections.

These results confirm the good penetration of fluoroquinolones into ocular tissues and highlight potential differences that may be observed when using pigmented or albino rabbits.

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