European Journal of Ophthalmology / Vol. 16 no. 2, 2006 / pp. 311-317

Rufloxacin eyedrops: Effect of different formulations on ocular pharmacokinetics in rabbits

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PURPOSE. *To evaluate the aqueous humor pharmacokinetics of rufloxacin in rabbits after topical administration of different formulations, and to individuate the ones showing the best pharmacokinetic profile.*
METHODS. *Six formulations were instilled in rabbit eyes: two pH 7.2 suspensions of non-salified rufloxacin base, or zwitterion (RUF), one of which was viscosized with tamarind seed polysaccharide (TSP); two pH 7.2 solutions of RUF obtained using hydroxypropyl-β-cyclodextrin (CD), one of which was viscosized with TSP; and two pH 5.0 solutions of rufloxacin hydrochloride (RUF-HCl), one of which was viscosized with TSP. At different times after administration, samples of aqueous humor were withdrawn and analyzed by high-pressure liquid chromatography. The main pharmacokinetic parameters of RUF in the aqueous humor produced by the different formulations were calculated and statistical differences were assessed.*
RESULTS. *The best results, in terms of aqueous humor bioavailability, were observed with two TSP-viscosized formulations: a solution of the hydrochloride (TSP/RUF-HCl) and a suspension of the base (TSP/RUF), followed by the non-viscosized solution of RUF-HCl. The formulations containing CD-solubilized RUF were much less effective.*
CONCLUSIONS. *The present data confirm the significant availability-enhancing properties of tamarind seed polysaccharide, and indicate that solubilization of RUF with hydroxypropyl-β-cyclodextrin (CD/RUF) results in decreased drug availability with respect to standard formulations. Two of the TSP-viscosized formulations (RUF suspension and RUF-HCl solution) produced aqueous humor RUF concentrations in the range of activity against Enterobacteriaceae and Pseudomonas aeruginosa, thus warranting further studies on applications of rufloxacin in ocular therapy. (Eur J Ophthalmol 2006; 16: 311-7)*

KEY WORDS. *Rufloxacin hydrochloride, Rufloxacin base, Tamarind seed polysaccharide, Hydroxypropyl-β-cyclodextrin, Ocular pharmacokinetics, Rabbits*

Accepted: September 23, 2005

INTRODUCTION

Clinical studies have shown that treatment of ocular bacterial infections with fluoroquinolone monotherapy (particularly ciprofloxacin, ofloxacin, lomefloxacin, and norfloxacin) can be favorably compared with conventional combined antibiotics therapy (1- 4). Since therapeutic concentrations of fluoroquinolones are reached in the aqueous humor after topical administration (5-7), these agents can be usefully employed for treatment of internal ocular infections.

Rufloxacin, a third-generation fluoroquinolone, is characterized by a broad spectrum of activity against gram-positive and Gram-negative aerobic bacteria and by fa-

1120-6721/311-07$15.00/0

vorable pharmacokinetic properties, such as rapid absorption, long serum half-life, good tissue penetration, high and prolonged concentrations in tissues and body fluids, and high tolerability (8, 9). This drug, however, seems to have been little investigated in topical ocular therapeutics. Nucci et al (10) have reported on rufloxacin pharmacokinetics in rabbit plasma, aqueous, and vitreous after a single intravenous administration. More recently, Ghelardi et al investigated the intra-aqueous penetration of rufloxacin in rabbit eyes after ocular administration of the drug in association with tamarind seed polysaccharide (TSP), a mucoadhesive polymer (11), and reported that the polysaccharide significantly increased the intra-aqueous penetration of the drug both in infected and non-infected eyes.

Aim of this investigation was to evaluate the aqueous humor pharmacokinetics of rufloxacin in rabbits after topical administration of different formulations, in order to individuate the ones showing the best pharmacokinetic profile. Rufloxacin, in analogy with other fluoroquinolones (12), is water-soluble as the hydrochloride (RUF-HCl), yielding relatively acidic solutions (pH ≤ 5.0) which are potentially irritant to ocular tissues, whereas it is insoluble as the non-salified base (RUF). It is worth nothing that the drug is an amphoteric molecule, or zwitterion (i.e., it carries both a positive and a negative charge in the molecule), with isoelectric point at pH 7.15 (13). Due to its insolubility, RUF was therefore formulated as a suspension in a pH 7.2 medium, more closely representing physiologic conditions, while RUF solutions at pH 7.2 were prepared using hydroxypropyl-β-cyclodextrin (HP-β-CD), a cyclic oligosaccharide capable of forming inclusion complexes with lipophilic drugs. HP-β-CD in our previous studies (14) had proven a more efficient solubilizer with respect to other cyclodextrins. Incidentally, one cyclodextrin is used as solubilizer for diclofenac in Voltaren Ophtha CD eyedrops (Novartis Ophthalmics AG, Switzerland). TSP was also added to some of the present formulations, in order to prolong the contact time with the corneal/conjunctival epithelium, thereby increasing the drug bioavailability (11, 15, 16).

MATERIALS AND METHODS

Materials

100% Crystalline rufloxacin hydrochloride (RUF-HCl) was provided by Dong HWA Pharmaceutical Company (Seoul, Korea). Rufloxacin base, or zwitterion (RUF), was precipitated from a RUF-HCl solution by adjusting its pH to 7.8. The collected precipitate was washed with water, dried in vacuo, and purified by crystallization from ethanol. 2-Hydroxypropyl-β-cyclodextrin DS 0.61 (HP-β-CD) was purchased from Roquette (Lestrem, France). Tamarind seed polysaccharide (TSP) was obtained from Farmigea SpA (Pisa, Italy). All other chemicals used were of pharmaceutical or analytical grade. Doubly distilled water was used throughout the study.

Test formulations

The formulations (Tab. I) contained either rufloxacin base (RUF) at pH 7.2 or rufloxacin hydrochloride (RUF-HCl) at pH 5.0. The drug content was in all cases 0.3% (w/v) as base, corresponding to the concentration of other commercially available ophthalmic fluoroquinolones, e.g., ciprofloxacin (Ciloxan; Alcon Laboratories Inc.) or ofloxacin (Ocuflox; Allergan, Inc.). HP-β-CD was added as solubilizer to RUF only, since RUF-HCl is water soluble. The amount of HP-β-CD needed to solubilize 0.3% (w/v) RUF was determined by solubility studies, performed according to the method of Higuchi and Connors (17).

The RUF suspensions (S-RUF, TSP/RUF) were prepared

TABLE I - RUFLOXACIN FORMULATIONS TESTED IN THE STUDY

Test formulations*	Type	Drug	pH	HP-β-CD %w/v	TSP %w/v
S-RUF	Suspension	RUF	7.2	—	—
CD/RUF	Solution	RUF	7.2	8.6	—
TSP/RUF	Suspension	RUF	7.2	—	1.0
TSP/CD/RUF	Solution	RUF	7.2	8.6	1.0
RUF-HCl	Solution	RUF-HCl	5.0	—	—
TSP/RUF-HCl	Solution	RUF-HCl	5.0	—	1.0

*All formulations contained 0.3 % (w/v) rufloxacin base (RUF).
HP-β-CD = Hydroxypropyl-β-cyclodextrin; TSP = Tamarind seed polysaccharide

by adding to pH 7.2, 0.1 M phosphate buffer, in the absence or in the presence of TSP, a 0.3 % (w/v) amount of finely ground drug base, then by sonicating for 25 min, a period sufficient to achieve saturation solubility (UP200H ultrasonic processor, Dr. Hielscher GmbH, Teltow, Germany). Particle size, determined by microscopy, complied with the specifications of the European Pharmacopeia (Ph. Eur. 4th Ed.).

The cyclodextrin-RUF solutions (CD/RUF, TSP/CD/RUF) were obtained by dissolving RUF in pH 7.2, 0.1 M phosphate buffer containing the appropriate amount of HP-β-CD (14), with or without TSP.

The RUF-HCl solutions (RUF-HCl, TSP/RUF-HCl) were prepared by dissolving 0.33% (w/v) RUF-HCl (corresponding to 0.3 % w/v RUF) in distilled water, and adjusting the pH of the solution to 5.0 with 0.1 N NaOH. All formulations were made isotonic with NaCl (H. Roebling micro-osmometer, Berlin, Germany).

Animals

Male New Zealand albino rabbits, 2.8–3.5 kg (Pampaloni rabbitry, Fauglia, Italy), were used and treated as prescribed in the publication *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 92-93, revised 1985). All experiments were carried out under veterinary supervision, and the protocols were approved by the ethical-scientific committee of the University of Pisa.

The animals were housed singly in standard cages, at 19±1°C and 50±5% R.H., in a light-controlled room with 14 h light (6:00 am to 8:00 pm) and 10 h dark cycle with no restriction of food or water. During the experiments the rabbits were placed in restraining boxes: they were allowed to move their heads freely, and their eye movements were not restricted.

Ocular administration of rufloxacin in rabbits

One hundred microliters (2 x 50 µL, with 3-min interval) of the formulations under study were carefully instilled in the lower conjunctival sac of one eye of each rabbit. At least six rabbits were used for each time point; each animal was tested at one time point only. At times = 15, 30, 45, 60, 90, and 120 min after administration, the rabbits' eyes were anesthetized by locally applying 20 µL of 0.4% oxibuprocaine hydrochloride, and 50–80 µL of aqueous humor were aspirated from the anterior chamber using a 1.0-mL insulin syringe fitted with a 29-gauge needle (B-D, Micro-Fine U-40 insulin, Beckton Dickinson, Dublin, Ireland).

The aqueous humor samples were concentrated to dryness and stored at -18°C. For analysis, the samples were submitted to vortex agitation after addition of 100 µL of distilled water and 400 µL of dichloromethane. After centrifugation (10 min at 4000 rpm), the organic phase was collected and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in a mixture acetonitrile/0.025 M phosphoric acid (89:11 v/v) and analyzed by HPLC.

HPLC analysis

HPLC with fluorescence detection was used to measure the RUF concentration in the aqueous humor, according to a modification of the method of Beck et al (18). Reversed phase chromatography was performed on a Waters 600E liquid chromatography equipment (Waters, Milford, MA, USA) with a 7725 Rheodyne injection valve and a Waters 600E spectrofluorimetric detector. The chromatograms were recorded by a 746 Waters Data Module.

The mobile phase consisted of acetonitrile/0.025 M phosphoric acid (89:11 v/v), adjusted to pH 3.0 with tetrabutyl ammonium hydroxide (40% water solution, Sigma Chemical Co., St. Louis, MO, USA). The isocratic flow rate of the mobile phase was 0.8 mL/min. The column was a Kromasil C18 (250 x 4.60 mm). The fluorescence detector was set for excitation at 294 nm and for emission at 521 nm.

The amount of RUF in the samples was determined by comparison with an appropriate standard curve, obtained by adding increasing amounts of RUF to pools of blank aqueous humor samples.

Determination of pharmacokinetic parameters and statistical analysis

The apparent elimination rate constants (K_e) of RUF from the aqueous humor were calculated from log [aqueous humor concentration] vs time linear regression plots; the mean residence time (MRT) was calculated from the ratio of the area under the first moment curve (AUMC, concentration and time vs time from t = 0 to 120 min) to AUC (area under the RUF concentration vs time curve), according to the following equation: MRT=AUMC/AUC (19, 20). The AUC and AUMC values were calculated using the linear trapezoidal rule (Kaleidagraph, Synergy Software, Reading, PA, USA).

The statistical significance of the differences between means of aqueous humor RUF concentration was evaluated using an analysis of variance test (StatView Software, Abacus Concepts Inc., Berkeley, CA, USA). The evaluation included calculation of means and standard errors (SE) and group comparison using the Fisher PLSD test. Differences were considered statistically significant at $p<0.05$.

RESULTS

The aqueous humor RUF concentration vs time profiles resulting from administration to rabbits of the formulations under study are presented in Figure 1; the data are reported as the mean ± SE (n=6). The pharmacokinetic parameters of RUF in the aqueous humor are summarized in Table II, and the AUC values (corresponding to the aqueous humor bioavailability) of all tested formulations are reported graphically in Figure 2.

A comparison of the two formulations based on RUF alone, without cyclodextrin or polymer, S-RUF and RUF-HCl, shows that the S-RUF suspension produced a peak concentration of 0.875±0.178 µg/mL within 30 min of administration, while the RUF-HCl solution showed a significantly higher C_{max} value (1.985±0.288 µg/mL) at the same t_{max}. The corresponding AUC values were 67.63 and 145.33, indicating a higher bioavailability of the drug (hydrochloride) as solution, which appeared well-tolerated by the animals' eyes. On the contrary, the concentration vs time profiles corresponding to the CD/RUF solution (cyclodextrin-solubilized RUF) and to the S-RUF suspension were superimposable until 60 min after administration, while differing slightly but significantly after 90 and 120 min, when CD/RUF produced higher aqueous humor levels.

Addition of 1.0% TSP (formulations 3, 4, and 6) increased noticeably the viscosity of the formulations, which exhibited a non-Newtonian, pseudoplastic rheologic behavior with apparent viscosity values of 127.87 and 18.2 mPa.s at shear rates of 25.6 and 3877.7 s^{-1}, respectively (15). Correspondingly, the TSP/RUF suspension and the TSP/RUF-HCl solution showed the highest concentration peaks (3.087 and 3.159 µg/mL) and a strong bioavailability increase, with AUC values 187.88±20.60 and 196.97±31.68 min µg/mL, respectively. As expected, the peak time of the suspension was delayed: 60 min after administration vs 45 min for the TSP/RUF-HCl solution, and its Ke value was remarkably higher with respect to those observed for the solution formulations (0.0276 min^{-1} for TSP/RUF vs 0.0088, 0.0095, and 0.0091 min^{-1} for TSP/CD/RUF, RUF-HCl, and TSP/RUF-HCl, respectively).

Viscosization of the CD/RUF solution with TSP to give TSP/CD/RUF, however, was not equally effective: the main pharmacokinetic parameters (C_{max}, AUC) of this formulation were even lower than those of the TSP-free corresponding formulation, and the lowest of the whole group of vehicles.

DISCUSSION

As stated in the Introduction, a 0.3% rufloxacin hydrochloride solution (RUF-HCl) is relatively acidic (pH 5.0) and potentially irritant to ocular tissues. The induced lacrimation might result in quick loss of the medication from the eye, possibly reducing ocular bioavailability. Two different approaches were followed to obtain rufloxacin eyedrops with a physiologic pH: either formulating the drug base (zwitterion) as a suspension (S-RUF), or dissolving it with the aid of hydroxypropyl-β-cyclodextrin (CD-RUF). Administration to rabbits of the RUF-HCl solution, in spite of its pH being 2 units lower with respect to

TABLE II - PHARMACOKINETIC PARAMETERS OF RUFLOXACIN IN THE AQUEOUS HUMOR OF ALBINO RABBITS (N=6)

Formulation	C_{max} (µg/mL±SE)	t_{max} (min)	AUC (min µg/mL±SE)	K_e (min^{-1})	MRT (min)
S-RUF	0.875±0.178	30	67.63±14.91	0.0119	83.75
CD/RUF	1.029±0.228	30	89.07±20.05	0.0166	60.17
TSP/RUF	3.087±0.468	60	187.88±20.60	0.0276	36.23
TSP/CD/RUF	0.260±0.053	45	24.48±6.27	0.0088	113.64
RUF-HCl	1.985±0.288	30	145.33±27.82	0.0095	105.26
TSP/RUF-HCl	3.159±0.291	45	196.97±31.68	0.0091	110.01

AUC = Area under the curve; MRT = Mean residence time; RUF = Rufloxacin base

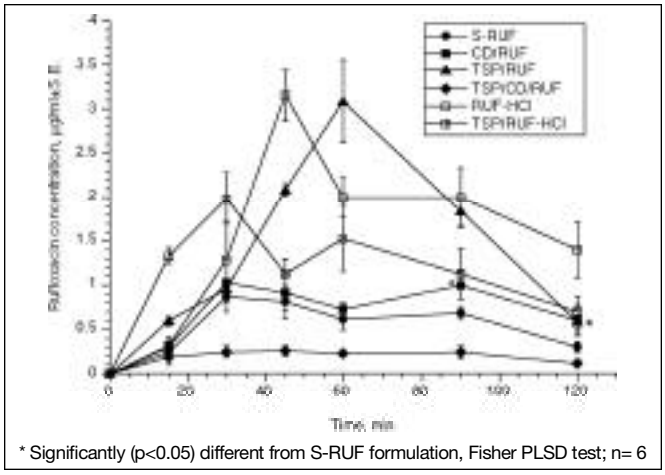

Fig. 1 - *Concentration profiles of rufloxacin in the aqueous humor of rabbits after administration of the formulations under study.*

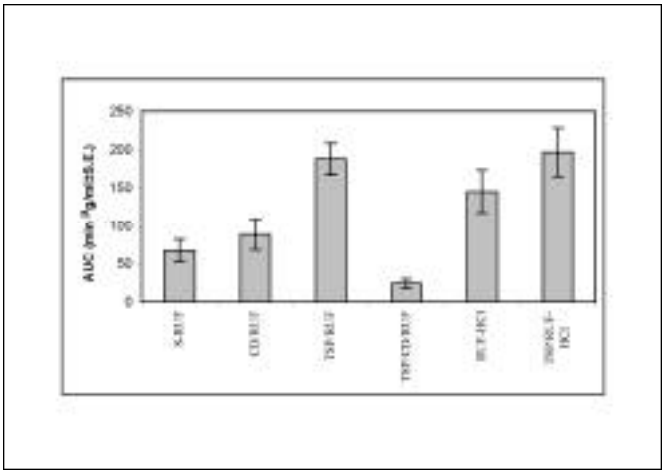

Fig. 2 - *Area under the curve values (corresponding to the aqueous humor availability) of the formulations under study (n=6).*

the physiologic value, produced a twofold greater bioavailability with respect to the pH 7.2 suspension of the zwitterion (S-RUF). A possible reason for the activity of the RUF-HCl solution, which appeared reasonably well tolerated, might reside in the buffering action of the rabbits' tear fluid, whose pH was reported to be 7.47, i.e., in the alkaline range (21, 22). A poor bioavailability, due to a slow rate of dissolution of the suspended drug, is an inherent disadvantage of ophthalmic suspensions, and can be obviated by reducing the particle size (thus increasing the dissolution rate) and/or by prolonging the time of residence of the medication in the eye (23). When the latter approach was followed by adding TSP to the S-RUF suspension, the resulting, viscosized suspension TSP/RUF showed a threefold AUC increase with respect to the original suspension (187.88 vs 67.63 min µg/mL). Interestingly, the pH 5.0 RUF-HCl solution also benefited, albeit to a lesser extent, by TSP addition and showed increased C_{max} and AUC values.

RUF solubilization with HP-β-CD yielded a pH 7.2 solution (CD-RUF), which showed a little, nonsignificant bioavailability increase with respect to S-RUF. As illustrated in Figure 1, this AUC increase was mainly due to significantly higher RUF concentrations in the aqueous humor 90–120 min after administration, when compared with the S-RUF suspension. As specified by different authors (24, 25), formation of drug/CD complexes may frequently result in decreased drug availability, particularly when the complex may not have enough time to release the drug before its clearance from the precorneal area.

A highly polar species such as RUF might not be able to partition out of the CD complex once in the corneal or conjunctival epithelium. Insufficient dilution of the complex can further contribute to a decreased drug availability. As specified by Stella et al (26), dilution, which is minimal when a drug/CD complex is administered ophthalmically, is likely to account for little, if any, drug dissociation from a CD complex. The further, strong bioavailability decrease observed after TSP addition to CD/RUF might be due to hindered dilution and to slow diffusion of RUF from CD-complex, caused by the viscous vehicle. Addition of water-soluble polymers (e.g., hydroxypropyl methylcellulose, sodium hyaluronate, polyacrylic acid) to CD-drug complexes is known to increase the solubilizing effect of CDs on water-insoluble drugs, consequently increasing the amount of drug available for penetration into the eye (27, 28), but this effect is not apparent with some drugs (29). An optimum polymer concentration (0.1–0.5%) and a low viscosity range (1.5–11 mPa.s) seem to be necessary to achieve such result (29). The higher polymer concentration (1.0%) used in the TSP/CD/RUF formulation, and its relatively high viscosity, may have in some way prevented an efficient release of RUF from the CD complex.

In conclusion, the present data, while indicating that the present CD/RUF complex shows a decreased drug availability with respect to standard formulations, confirm the significant availability-enhancing properties of tamarind seed polysaccharide. The TSP/RUF suspension and the TSP/RUF-HCl solution produced aqueous humor concentrations around 3 µg/mL, in the range of activity of RUF against Enterobacteriaceae and Pseudomonas aerugi-

nosa (MIC90 1–8 mg/L) (30). These findings warrant further studies on applications of rufloxacin in ocular therapy, also in consideration of the fact that this drug is active against Gram-positive bacteria (8, 9) responsible for 94.2% of postoperative growth isolates (31).

The authors have no commercial or proprietary interest in this article.

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