Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 173–176, 2002.

Formulation and Preliminary *in vivo* Testing of Rufloxacin-Cyclodextrin Ophthalmic Solutions

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(Received: 7 May 2002; in final form: 1 October 2002)

Key words: aqueous humor pharmacokinetics, β-cyclodextrin, γ-cyclodextrin, hydroxypropyl-β-cyclodextrin, hydroxypropyl methylcellulose, rabbits, rufloxacin, solubilization

Abstract

Aim of the present work was to investigate the effect of some cyclodextrins (CDs) on the solubility and ocular bioavailability of rufloxacin base (RUF), with the ultimate goal of developing an ophthalmic formulation. Phase solubility studies of RUF in pH 7.4 buffer were carried out in the presence of β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD). The effect of hydroxypropyl methylcellulose (HPMC) on RUF solubility was evaluated after heating the solutions containing HP-β-CD at 120 °C.

A significant enhancement of RUF solubility was achieved by associating the drug with CDs, particularly HP-β-CD. This CD formed with RUF a less stable complex than that formed by β-CD, but did not suffer the solubility limitations of the parent CD, and showed a higher solubilizing capacity than γ-CD. Addition of 0.25% (w/v) HPMC to solutions containing HP-β-CD increased the solubilizing effect of this CD, thus allowing reduction of the amount necessary for solubilization of 0.3% (w/v) RUF.

Preliminary pharmacokinetic data in rabbits indicated that the ocular bioavailability of 0.3% (w/v) RUF solubilized by HP-β-CD was higher when compared with a 0.3% (w/v) RUF suspension used as reference.

Introduction

Rufloxacin (RUF) is an oral fluoroquinolone characterized by a broad spectrum of activity against gram-negative and gram-positive aerobic bacteria. In view of its pharmacokinetic profile RUF can be used once-daily for treatment of urinary and respiratory tract infections [1]. The observation that some fluoroquinolones (norfloxacin, ofloxacin, ciprofloxacin and levofloxacin) are successfully used for topical treatment of ocular infections as 0.3% aqueous eyedrops [2], prompted the present authors to test RUF for the same purpose.

The formulation of aqueous RUF solutions, however, involves some problems, due to the poor water solubility of the drug (approximately 26 mg/100 mL at pH 7.4). It was speculated that RUF solubility may be improved by association with cyclodextrins (CDs), known to form water-soluble inclusion complexes with many compounds [3, 4]. Recent studies have shown that CDs are useful additives in ophthalmic formulations, since they may increase not only the aqueous solubility but also the stability and bioavailability of some drugs, while decreasing their irritant effect [5]. The addition of small amounts of water-soluble polymers has been reported to enhance the solubilizing effect of CDs [6], thus allowing reduction of the CD amount required for drug solubilization.

Aim of the present work was to investigate the effect of some CDs on the solubility and ocular bioavailability of RUF, with the ultimate goal of developing an ophthalmic formulation. Phase solubility studies of RUF in pH 7.4 buffer were carried out in the presence of β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD). These CDs were selected because they are considered safe upon ocular applications [7]. The effect of hydroxypropyl methylcellulose (HPMC) on RUF solubility was evaluated after heating the solutions containing HP-β-CD at 120 °C. The ocular bioavailability of RUF was assessed by determining the RUF concentration in the aqueous humor of rabbits, after administration of 0.3% (w/v) drug solubilized by HP-β-CD.

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Experimental

Chemicals

Rufloxacin-hydrochloride was provided by Dong HWA Pharmaceutical Company (Seul, Corea). Rufloxacin free base was precipitated from the salt solution by adjusting the pH at 8.5 with 1N NaOH. β-Cyclodextrin and 2-hydroxypropyl-β-cyclodextrin (DS 0.61) were purchased from Roquette (Lestrem, France), whereas γ-cyclodextrin was a commercial sample from Cyclolab (Budapest, Hungary). Hydroxypropyl methylcellulose 4000 was obtained from Prodotti Gianni (Milan, Italy). All other chemicals used were of pharmaceutical or analytical grade. Double distilled water was used throughout the study.

Solubility studies

Solubility studies on RUF were performed according to the Higuchi and Connors method [8]. An excess amount of drug (20 mg) was added to 5 mL portions of pH 7.4 (0.1 M) phosphate buffer containing variable CD amounts (from 1×10^{-3} to 1.4×10^{-2} M of β-CD; from 3.61×10^{-3} to 7.23×10^{-2} M of HP-β-CD; from 7.23×10^{-3} to 7.23×10^{-2} M of γ-CD). The suspensions were mechanically shaken in a water bath at 25 °C until equilibrium was reached (at least 5 days). Solubility studies of RUF were also carried out adding HPMC (0.1–0.7 % w/v) to the suspensions containing HP-β-CD. In this case the suspensions formed were heated in an autoclave in sealed containers to 120 °C for 20 min, then were allowed to equilibrate in the shaking bath at 25 °C for 5 days. Separate experiments showed that this period of time was sufficient, since longer equilibration times (up to 20 days) did not result in further drug precipitation. The pH of the suspensions was monitored during equilibration and adjusted to 7.4 with NaOH, if necessary. The suspensions were filtered and suitably diluted with pH 7.4 phosphate buffer for analysis. The RUF content was determined by UV spectrophotometry (Shimadzu UV-1204 spectrophotometer) at 245 nm. The presence of CDs and of HPMC did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate; the coefficient of variation associated with each measurement was never greater than 3%.

Ocular bioavailability studies

The aqueous humor pharmacokinetics of RUF were investigated on male, non anaesthetised New Zeland albino rabbits (3.0–3.5 Kg, Pampaloni Rabbitry, Fauglia, Italy). The animals were used and treated as prescribed in the publication "Guide for the care and use of laboratory animals" (NIH Publication Nos. 92–93, revised 1985), were allowed to move their heads freely, and their eye movements were not restricted. The following formulations were tested: (1) a 0.3% (w/v) RUF solution in pH 7.4 buffer containing 8.6% (w/v) HP-β-CD (HP-β-CD/RUF); (2) as reference, a 0.3% (w/v) RUF suspension in pH 7.4 buffer (S-RUF). The formulations were made appropriately isotonic with NaCl (H. Roebling micro-osmometer, Berlin, Germany).

The study was carried out by administering 100 μL (2×50 μL) of the formulations in the lower conjunctival cul-de-sac of rabbit eyes. At appropriate times after administration the rabbits were anaesthetised (i.m. administration of 30 mg kg^{-1} ketamine (Inoketam 1000 solution, Virbac S.r.l., France) and 5.0 mg kg^{-1} xylazine (Rompum 2% solution, Bayer AG, Leverkusen, Germany), and 60–80 μL of aqueous humor were aspirated from the anterior chamber using a 1.0 mL syringe fitted with a 29G needle (Micro-Fine, Beckton Dickinson, Dublin, Ireland). At least four rabbits (four eyes) were used for each time point and for each formulation. The aqueous humor samples were dried *in vacuo* and stored until analysis.

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.* [9]. Reversed phase chromatography was performed on a Waters 600E liquid chromatography equipment with a 7725 Rheodyne injection valve and a Waters 600E spectrofluorimetric detector. The chromatograms were recorded by a 746 Data Module (Waters).

For analysis, the aqueous humor samples were added of 100 μL of distilled water and 400 μL dichlormethane, then were agitated in an overhead shaker for 10 min. After centrifugation (10 min at 4000 r.p.m.), 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); 60 μL of the solutions were injected into the column.

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). The isocratic flow rate of the mobile phase was 0.8 mL/min. The column was a Kromasil C18 (250×4.6 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.

Results and discussion

Solubility studies

The phase solubility plots of RUF in aqueous CD solutions at pH 7.4 are shown in Figure 1. According to the Higuchi and Connors classification [8], the diagrams obtained for the three CDs under study were of A_L-type, since the drug solubility increased linearly as a function of CD concentration. The apparent stability constant of the drug-CD complexes, assuming a 1:1 stoichiometry ($K_{1:1}$), were calculated from the slope of the phase solubility diagrams using the Higuchi and Connors Equation (1):

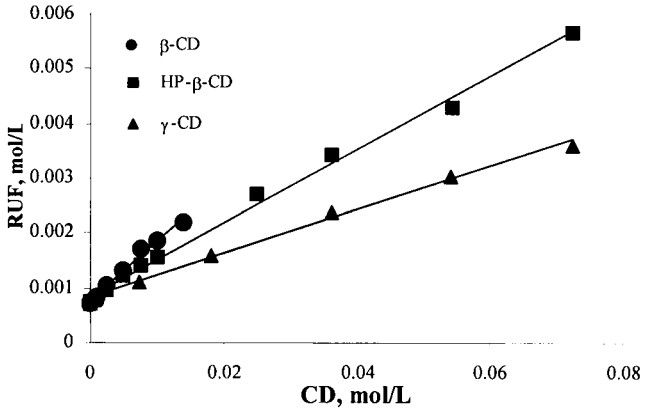

Figure 1. Phase solubility diagrams of RUF at increasing amounts of the different CDs in pH 7.4 phosphate buffer (mean of three experiments, CV < 3%, error bars omitted for clarity).

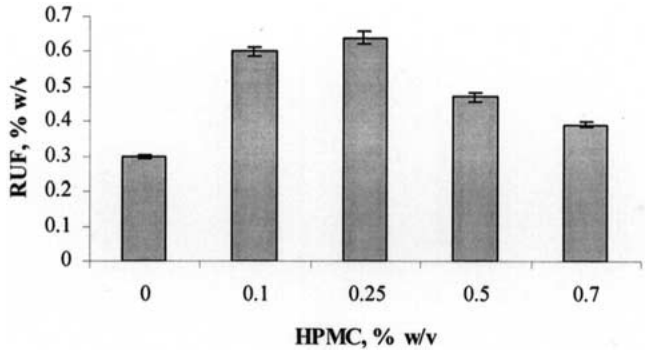

Figure 2. Effect of different concentrations of HPMC on RUF solubility in aqueous, pH 7.4, 8.6% (w/v) HP-β-CD solutions, after heating at 120 °C (mean of three experiments, CV < 3%).

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the intrinsic solubility of RUF at pH 7.4 (7.3×10^{-4} M). The calculated $K_{1:1}$ value was 139 M^{-1} with β-CD, 95 M^{-1} with HP-β-CD and 48 M^{-1} with γ-CD. The stability constant of RUF/γ-CD complex is lower than those of the complexes with β-CDs, suggesting that the β-CD molecule has a higher affinity for the RUF molecule when compared to γ-CD. The $K_{1:1}$ values of the RUF complexes with β-CD and HP-β-CD appeared slightly different, the one with the parent CD being higher. However, higher solubility enhancement was achieved with HP-β-CD, since β-CD has a limited aqueous solubility. According to the phase solubility diagram, the amount of HP-β-CD needed to solubilize 0.3% (w/v) RUF in pH 7.4 buffer was ~8.6% (w/v). Although this HP-β-CD concentration is not very high, its reduction was desirable for a variety of reasons, including toxicological considerations, isotonicity adjustment, drug bioavailability and production costs.

According to recent reports [5] water-soluble polymers and particularly HPMC [10] when activated by heating may enhance drug solubilization induced by CDs. Accordingly, the solubility of RUF was determined in the presence of varying HPMC concentration (from 0.1% to 0.7% w/v) after heating the solutions in autoclave at 120 °C for 20 min. It is worth noting that these heating conditions are usually

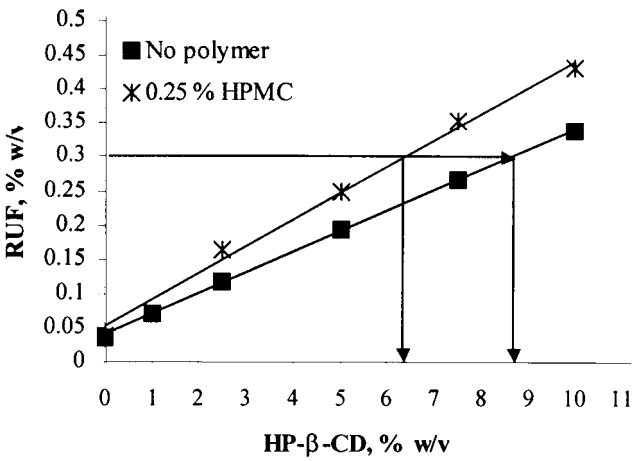

Figure 3. Phase solubility diagram of RUF in aqueous, pH 7.4, HP-β-CD solutions containing 0.25% (w/v) HPMC, after heating at 120 °C. The phase solubility diagram of the drug in the absence of HPMC is also shown for comparison (mean of three experiments, CV < 3%, error bars omitted for clarity).

employed to sterilize pharmaceutical preparations. The solubility of RUF in 8.6% (w/v) aqueous HP-β-CD solutions at pH 7.4, heated in autoclave in the presence of increasing concentrations of HPMC, is illustrated in Figure 2. The addition of the water-soluble polymer increased the solubilizing effect of HP-β-CD, the 0.1% and 0.25% HPMC concentrations providing the highest increases in drug solubility. The 0.25% HPMC was considered suitable for an ophthalmic RUF formulation, since it also induced a slight viscosity increase of the solution. Therefore, a phase solubility diagram of RUF in HP-β-CD solutions in pH 7.4 buffer containing 0.25% (w/v) HPMC, heated in autoclave at 120 °C for 20 min, was constructed (Figure 3). The $K_{1:1}$ of the complex calculated from the A_L type diagram by the Higuchi and Connors Equation (1) was 111 M^{-1}. This value was slightly higher than the one obtained in the absence of the polymer. As a consequence, in the presence of 0.25 % (w/v) HPMC the amount of HP-β-CD needed to solubilize 0.3% (w/v) RUF in pH 7.4 buffer decreased to 6.4% (w/v).

Ocular bioavailability studies

The results of preliminary ocular bioavailability studies in rabbits are shown in Figure 4, illustrating the aqueous humor RUF concentration profiles *vs* time, and in Table 1, where the following pharmacokinetic parameters are reported: peak time (T_{max}, min), RUF concentration peak (C_{max}, μg/mL), area under the concentration *vs* time curve (AUC, min μg/mL). The AUC values were calculated from the beginning (t_0) to the end of the observation time (t_{last}) from the graphs using the linear trapezoidal rule (Kaleidagraph, Synergy Software).

The HP-β-CD/RUF solution produced a peak concentration of 1.12 μg/mL within 30 min of administration, increased with respect to the reference S-RUF suspension (0.81 μg/mL). The HP-β-CD/RUF solution produced significantly higher RUF concentrations in the aqueous humor 90–120 min after administration when compared with the

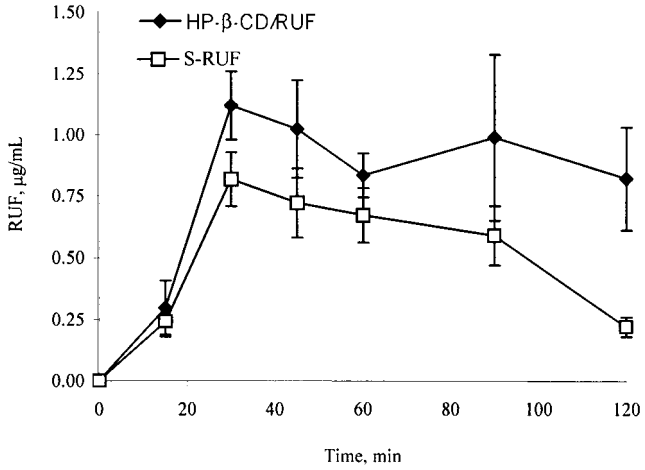

Figure 4. RUF aqueous humor concentration vs time profiles after administration of the formulations under study (means ± S.E., $n = 4$).

Table 1. Pharmacokinetic parameters calculated after ocular administration in rabbits of the formulations under study. (Means ± S.E., $n = 4$)

Formulations	T_{max} (min)	C_{max} (μg/mL)	AUC (min μg/mL)
HP-β-CD/RUF	30	1.12 ± 0.14	97.48 ± 22.12
S-RUF	30	0.81 ± 0.11	62.86 ± 11.34

reference suspension, and the AUC values confirmed the greater bioavailability (about 1.5-fold) of the HP-β-CD/RUF solution. This increased bioavailability was presumably not due to an increased viscosity of the solution, that showed a (Newtonian) viscosity value of 1.25 cP.

Conclusions

A significant enhancement of RUF solubility has been achieved by associating the drug with CDs, particularly HP-β-CD. This CD formed with RUF a less stable complex than β-CD, but did not suffer the solubility limitations of the parent CD, and displayed a higher solubilizing ability with respect to γ-CD.

Addition of 0.25% HPMC to solutions containing HP-β-CD increased the solubilizing effect of this CD, allowing a reduction of the amount necessary for solubilization of RUF.

Preliminary pharmacological data on rabbits indicated that ocular administration of RUF solubilized with HP-β-CD ensured a higher drug concentration in the aqueous humor when compared with a RUF suspension. The *in vivo* testing of 0.3% (w/v) RUF formulation containing 6.4% (w/v) HP-β-CD and 0.25% (w/v) HPMC is under way.

Acknowledgments

The financial support of MIUR (PRIN) is gratefully acknowledged.

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