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Effect of chitosan on in vitro release and ocular delivery of ofloxacin from erodible inserts based on poly(ethylene oxide)

G. Di Colo *, Y. Zambito, S. Burgalassi, A. Serafini, M.F. Saettone

Department of Bioorganic Chemistry and Biopharmaceutics, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy

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Abstract

The effects of chitosan hydrochloride (CH–HCl) on in vitro release of ofloxacin (OFX) from mucoadhesive erodible ocular inserts and on the relevant ocular pharmacokinetics have been studied both to contribute evidence of the ability of CH–HCl to enhance transcorneal penetration of drugs and to increase the therapeutic efficacy of topically applied OFX. Circular inserts of 6 mm in diameter, 0.8–0.9 mm in thickness and 20 mg in weight, medicated with 0.3 mg drug, were prepared by powder compression. The addition of 10, 20 or 30% medicated CH–HCl microparticles, obtained by spray-drying, to formulations based on poly(ethylene oxide) of MW 900 kDa (PEO 900) or 2000 kDa (PEO 2000) produced changes in the insert microstructure which accelerated both insert erosion and OFX release from inserts. The effect was stronger with higher CH–HCl fractions. Of the CH–HCl-containing formulations based on either PEO 900 or PEO 2000, PEO 900–CH–HCl (9:1 w/w) was more suitable for a prolonged OFX release. Following insertion in the lower conjunctival sac of the rabbit's eye, such an insert produced no substantial increase of AUC_{eff} (AUC in the aqueous humour for concentrations $> MIC_{90\%}$) with respect to inserts based on plain PEO; however, it produced a concentration peak in the aqueous significantly higher than that produced by any of the CH–HCl-free PEO inserts, and well higher than the $MIC_{90\%}$ for the more resistant ocular pathogens (7 μg/ml vs. 4 μg/ml). It has been argued that the increase was due to the ability of CH–HCl to enhance the transcorneal permeability of the drug.

Keywords: Ofloxacin; Chitosan; Poly(ethylene oxide); Erodible ocular insert; Ocular drug delivery; Transcorneal penetration enhancement

1. Introduction

Of the topically applied broad spectrum fluoroquinolone antibiotics, ofloxacin (OFX) can better penetrate through the corneal barrier into the aqueous humour (Beck et al., 1999) and, therefore, is more suitable for a topical treatment of endophthalmitis. In fact, following application of 0.3% OFX eyedrops, the concentration of the antibiotic in the aqueous humour reached values higher than the $MIC_{90\%}$ of the frequently occurring gram-positive and -negative bacteria (Beck et al., 1999; Cekic et al., 1999). A mean peak concentration of 5.25 μg/ml, higher than the

* Corresponding author. Tel.: +39-50-24000; fax: +39-50-43321

E-mail address: giadic@farm.unipi.it (G. Di Colo).

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$MIC_{90\%}$ for the more resistant ocular pathogens (4 μg/ml, according to Taravella et al., 1999), was found in the aqueous humour of the rabbit's eye following topical application of an erodible insert based on poly(ethylene oxide) (PEO), containing a 0.3 mg drug dose (Di Colo et al., 2001a). Application of such an insert increased bioavailability and peak concentration in the aqueous around 11 and 4 times, respectively, with respect to instillation of the same dose by the traditional 0.3% eyedrops. The increases were due to an erosion-controlled drug release from the insert that substantially limited the dose fraction cleared from the precorneal area by tear fluid drainage. Also it was speculated that the mucoadhesive PEO might enhance the corneal permeability of the drug (Di Colo et al., 2001a). This paper describes an attempt to further enhance OFX penetration into the aqueous humour by co-formulating the PEO insert with chitosan (CH). This cationic polymer has raised increasing interest as a component of ophthalmic preparations by virtue of its excellent ocular compatibility and its ability to interact with the negatively charged conjunctiva and cornea (Felt et al., 1999). Also, CH was found to enhance the permeability of intestinal and nasal epithelia by opening the tight junctions, thereby favouring paracellular drug transport (Artursson et al., 1994; Illum et al., 1994). The above information has suggested that CH may act as an enhancer of transcorneal drug penetration. This work aims at substantiating this hypothesis, and at exploiting the expected CH effect to increase the therapeutic efficacy of OFX, topically applied by the controlled-release PEO-based inserts. To this purpose, medicated chitosan hydrochloride (CH–HCl) microspheres have been added to the insert formulation and their effects on drug release mechanism from insert and drug penetration into the aqueous humour of the rabbit's eye have been studied.

2. Materials and methods

2.1. Materials

OFX and CH (minimum 85% deacetylated) were purchased from Sigma (Milan, Italy). PEO of different molecular weights were donated by Union Carbide Italia S.r.l. (Milan, Italy): PEO 900 (Polyox® WSR 1105, MW 900 kDa); PEO 2000 (Polyox® WSR N-60K, MW 2000 kDa). Buffer substances and all other chemicals or solvents were of reagent grade.

2.2. Characterisation of commercial CH

Commercial CH was characterised by determining its molecular weight by capillary viscometry (Ostwald capillary viscometer, Series 200) and its deacetylation degree by IR spectroscopy (Mattson 3000 FTIR spectrophotometer), following the procedures described by Khalid et al. (1999). An average viscometric molecular weight of 1460 kDa and a deacetylation degree of 89.9% resulted from the analysis. The water content of commercial CH, as determined by desiccation at 100 °C, was 12.3%.

2.3. Preparation of CH–HCl microspheres

CH was converted into CH–HCl by making an aqueous CH suspension to pH 4.7 with 1 N HCl. Following filtration to remove traces of non-dissolved material, a small aliquot of solution was evaporated to dryness and the residue was weighed, after equilibration with the ambient, to determine the CH–HCl concentration, and then the solution was diluted to a concentration of 0.5%. Medicated CH–HCl microspheres of <2.5 μm in diameter, as observed by an optical microscope, were prepared by spray-drying 0.5% CH–HCl solutions containing OFX concentrations calculated to obtain the designed payloads of 5, 7.5 and 15% (Mini Spray Dryer BÜCHI B-191, inlet and outlet air temperatures, 160 and 75 °C, respectively; spray nozzle, 0.7 mm; feed flow, 8 ml/min). Following preparation, the microspheres were allowed to equilibrate with the ambient before use (water content, around 11%). The actual payloads were determined spectrophotometrically at 286 nm, following dispersion of exactly weighed microsphere amounts in pH 7.4, 0.0026 M phosphate buffer and filtration (pore size, 0.45 μm).

2.4. Preparation of inserts

After passing through a 106 μm sieve, the PEO powder was thoroughly mixed with the CH–HCl microspheres, and then the mix was compressed by a hydraulic press (applied force, 9800 N) into flat-faced tablets of 6 mm diameter, 0.8–0.9 mm thickness and 20 mg weight. The inserts were formulated with 10.2, 20.8 and 31.6 wt.% fractions of CH–HCl microspheres containing 14.73, 7.22 and 4.74% OFX, respectively, so that the virtual PEO–CH–HCl wt. proportions were 9:1, 8:2 and 7:3, and in all cases the drug fraction and dose were 1.5% and 0.3 mg, respectively. For in vivo tests to be described in Section 2.7, PEO 900–CH–HCl (9:1) inserts containing sodium fluorescein as a tracer were prepared using PEO 900 into which 0.5% tracer had been dispersed by wetting the PEO powder portionwise with the appropriate volume of a 0.05% (w/v) sodium fluorescein solution in absolute ethanol, while mixing and letting the solvent evaporate, then vacuum-drying to a constant weight.

2.5. Measurement of drug release and insert erosion kinetics

An already described technique was used (Di Colo et al., 2001a). Briefly, each insert was tightly inserted into a 3 mm deep cylindrical cavity, of exactly the same diameter as the insert, bored at the centre of a 4 mm thick Teflon disk. Two disks, each containing an insert, were immersed, with the exposed insert surface in upward position, into 50 ml of 0.0026 M, pH 7.4 isotonic phosphate buffer, thermostated at 37 °C and stirred under controlled hydrodynamics. To determine the drug release kinetics, at interval samples of dissolution medium were spectrophotometrically analysed for the drug at 286 nm, after filtering (pore size, 0.45 μm). To determine the insert erosion kinetics, after a pre-established elution time each disk was withdrawn, dried and weighed, and the dissolved insert wt. fraction was computed. This procedure was repeated for different elution times.

2.6. Measurement of kinetics of water vapour absorption into inserts

The effect of CH–HCl on the hydration rate of inserts was assessed by determining the kinetics of water vapour absorption into inserts containing different CH–HCl wt. fractions. Each insert, contained in a small stainless steel wire mesh bag, was introduced into an air-tight glass chamber saturated with water vapour at 37 °C. The bag rested on a wire screen fixed at a small distance from the water surface. At 10 min intervals, the percent weight increase of insert was determined.

2.7. Animal tests

Tests of biocompatibility and residence time of inserts in the precorneal area, and measurement of OFX transcorneal penetration were carried out following the procedures described in detail in a previous paper (Di Colo et al., 2001a). An outline is given below.

Male, New Zealand albino rabbits of 2.5–3.0 kg were used. They were 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. Biocompatibility and residence time in the precorneal area of the PEO 900–CH–HCl (9:1) insert containing sodium fluorescein as a tracer were evaluated as follows. The insert was applied in the lower conjunctival sac of each eye of at least two rabbits. Following insertion, the device formed a superficial gel and adhered to the application site within 5 min. At appropriate time intervals, the state of the release system was observed in order to assess the time for complete insert gelation (TG) and the whole residence time of system in the precorneal area (TR). The checking intervals were regulated on the basis of the process rate, taking care that the last interval, during which insert gelation or gel dissolution was completed, was no longer than 10% of the assessed TG or TR value. Irritation signs, such as conjunctival/corneal edema and/or hyperaemia were checked, as well as fluorescence at the rabbit's

nose, due to lacrimation. For the measurement of OFX transcorneal penetration, the PEO 900–CH–HCl (9:1) insert, containing a nominal dose of 0.3 mg OFX, was applied in the lower conjunctival sac of one eye of each rabbit. After a pre-established time from administration, the rabbits were anaesthetised, and then 50–80 μl of aqueous humour was aspirated from the anterior chamber. At least six animals were used for each time point. The aqueous humour samples were immediately frozen and stored at −18 °C. For analysis, each sample was mixed with an equal volume of acetonitrile, then it was centrifuged and 20 μl of the supernatant was analysed by HPLC. The HPLC apparatus (Perkin-Elmer) consisted of Series 4 pump, 20 μl Rheodyne injector, LC 290 UV detector and 1020 LC Plus integrating system. The column (Macherey-Nagel 250 × 4 mm, Düren, Germany) was packed with Nucleosil® 100-5 C_{18} (5 μm). The mobile phase (flow rate 1.0 ml/min) was methanol–acetonitrile–citric acid 0.4 M (3:1:10). The UV detection was set at 294 nm. The OFX retention time was 6.8 min. The limit of quantitation was 0.12 μg/ml. The area under the concentration in the aqueous humour vs. time curve and over the level of 0.5 μg/ml ($MIC_{90\%}$ for the less resistant ocular pathogens; Taravella et al., 1999; see Fig. 6), coded AUC_{eff}, was calculated by the linear trapezoidal rule (Kaleidagraph, Synergy Software). The pharmacokinetics determined for the PEO 900–CH–HCl (9:1) insert were compared with those for the PEO 400 or PEO 900 insert not containing CH–HCl, as determined previously (Di Colo et al., 2001b). Significance of differences was evaluated by the Student's *t*-test.

3. Results and discussion

3.1. Preparation of CH–HCl microspheres

The spray-drying process described in Section 2.3 proved to be a rapid and efficient technique for preparation of CH–HCl microspheres medicated with pre-established OFX wt. fractions. Indeed, a comparison of the actual microsphere payloads, namely 4.74, 7.22 and 14.73% (w/w), with the designed values of 5, 7.5 and 15% (w/w), respectively, indicates a high entrapment efficiency of the process.

3.2. Kinetic measurements in vitro

The kinetics of insert erosion and drug release were measured in vitro for PEO-based inserts containing different fractions of CH–HCl microspheres and compared with corresponding data for CH–HCl-free inserts, in order to gain information on the release mechanism and on the insert formulation more suitable to evidence a possible corneal penetration enhancing effect of CH–HCl. Figs. 1 and 2 show that in the presence of CH–HCl microspheres in PEO 900 or PEO 2000 inserts, the insert erosion rate was increased, the effect being stronger with higher CH–HCl fractions. Since polymer dissolution must be preceded by hydration and swelling to a degree sufficient to allow disentanglement of polymer chains, acceleration of dissolution might be a consequence of an acceleration of hydration caused by CH–HCl. Indeed, the kinetics of water vapour absorption into inserts, represented in Fig. 3, shows that increasing CH–HCl wt. fractions increase the insert hydration rate. Following hydration, CH–HCl could interact with PEO, thus favouring chain disentanglement and dissolution. In fact, interchain interactions between CH and PEO have been reported (Khalid et al., 1999). In the presence

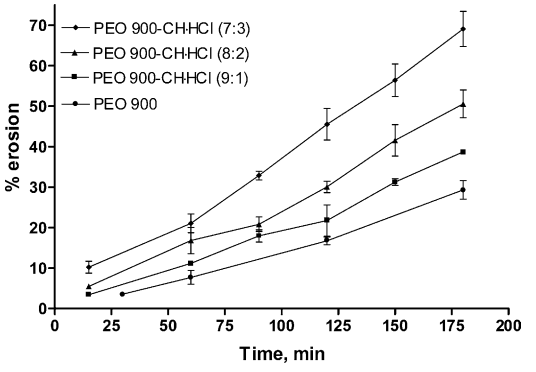

Fig. 1. Effect of varying CH–HCl fractions on the erosion kinetics of inserts based on PEO 900. Each data point is the mean ± S.D. of at least three values. The data for plain PEO 900 have been reproduced for comparison from Di Colo et al. (2001b).

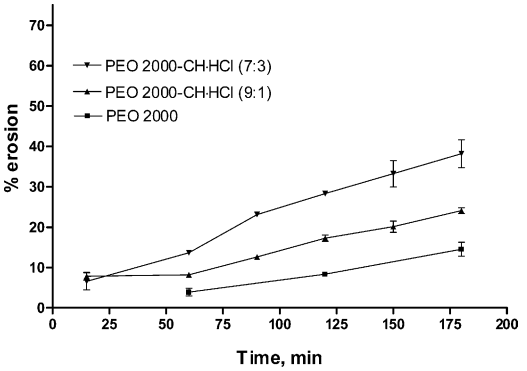

Fig. 2. Effect of varying CH–HCl fractions on the erosion kinetics of inserts based on PEO 2000. Each data point is the mean ± S.D. of at least three values. The data for plain PEO 2000 have been reproduced for comparison from Di Colo et al. (2001b).

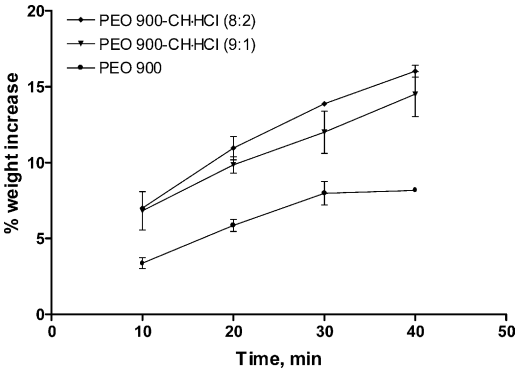

Fig. 3. Effect of varying CH–HCl fractions on the kinetics of water vapour absorption into inserts based on PEO 900. Each data point is the mean ± S.D. of at least three values.

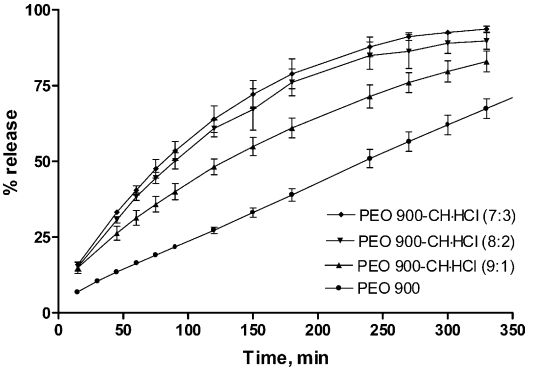

Fig. 4. Effect of varying CH–HCl fractions on release of 1.5% OFX from inserts based on PEO 900. Each data point is the mean ± S.D. of at least three values. The data for plain PEO 900 have been reproduced for comparison from Di Colo et al. (2001b).

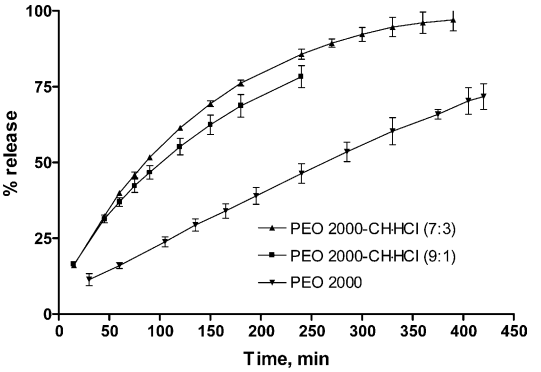

Fig. 5. Effect of varying CH–HCl fractions on release of 1.5% OFX from inserts based on PEO 2000. Each data point is the mean ± S.D. of at least three values. The data for plain PEO 2000 have been reproduced for comparison from Di Colo et al. (2001b).

of CH–HCl microspheres in PEO 900 or PEO 2000 inserts, drug release was also markedly accelerated, as Figs. 4 and 5 show. The effect can be quantified by comparing the values of t_{50}, i.e., the time for release of 50% dose, taken from the release plots of Figs. 4 and 5 and listed in Table 1. The enhancing effect of CH–HCl on drug release cannot solely be ascribed to the above-discussed acceleration of insert erosion. Indeed, if release had been erosion-controlled, fractional release should have been close to fractional erosion, at corresponding times, then, the values of percent insert eroded at t_{50}, taken from Figs. 1 and 2 and listed in Table 1, should be close to 50%. In fact, the values closer to 50% are those for the inserts based on pure PEO 400 and PEO 900, in the order. Indeed, a virtually erosion-controlled OFX release from such inserts has been substantiated in previous work (Di Colo et al., 2001a,b). In the presence of CH–HCl, on the other hand, the percent insert eroded at t_{50} was well below 50%, indicating that this polymer favoured a parallel release mechanism based on drug diffusion in matrix, by favouring matrix hydration and swelling. For a given CH–HCl fraction, the deviation from the erosive mechanism, as expressed by the

Table 1
In vitro data for inserts containing varying CH–HCl fractions: time for release of 50% dose (t_{50}) and percent insert eroded at t_{50}

Insert material	t_{50} (min)	% Eroded at t_{50}
PEO 400[a]	132	42
PEO 900[a]	240	35
PEO 900–CH–HCl (9:1)	131	25
PEO 900–CH–HCl (8:2)	90	20
PEO 900–CH–HCl (7:3)	82	29
PEO 2000[a]	266	25
PEO 2000–CH–HCl (9:1)	105	14
PEO 2000–CH–HCl (7:3)	86	21

[a] Data derived from Di Colo et al. (2001b).

deviation of the erosion values in Table 1 from 50%, was more marked with the inserts based on PEO 2000, as they eroded more slowly than those based on PEO 900, but released OFX at similar rates. Thus, the attempt to prolong release from CH–HCl-containing inserts by using PEO of higher molecular weight was unsuccessful. The t_{50} value in Table 1 relative to the PEO 900–CH–HCl (9:1) insert formulation is quite close to the value relative to the insert based on pure PEO 400, as derived from published data (Di Colo et al., 2001a). This suggested that a direct comparison of the in vivo behaviour of these inserts could provide evidence of a possible enhancing effect of CH–HCl on OFX transcorneal penetration. The inserts containing higher CH–HCl fractions were not used for the in vivo tests, since they released the drug at higher rates, less suitable for ocular inserts.

3.3. Animal tests

In Table 2, the behaviour of the PEO 900–CH–HCl (9:1) insert in the precorneal area is compared with that of the PEO 400 or PEO 900 insert not containing CH–HCl, as assessed previously (Di Colo et al., 2001b). Following insertion in the rabbit's eye, every insert type formed a superficial gel and adhered to the application site, and then the gel gradually spread over the cornea and eroded. As can be seen in Table 2, the mild irritation signs caused by the CH–HCl-containing insert were not more severe than those caused by the CH–HCl-free devices. Then, a fair ocular tolerability of CH–HCl can be admitted, although the possibility of some ocular irritation associated with the use of PEO-based inserts cannot be ruled out. The time for complete gelation of the PEO 900–CH–HCl (9:1) insert was shorter than that for the CH–HCl-free PEO 900 insert, in agreement with the water vapour absorption data, shown in Fig. 3, which point to a faster hydration of the former. Also the slightly shorter residence time of the PEO 900–CH–HCl (9:1) gel on the cornea compared to the pure PEO 900 gel agrees with the in vitro data in Fig. 1, showing a slightly faster erosion of the former.

The OFX concentration profiles in the aqueous, following administration of a 0.3 mg dose by the systems at comparison, are shown in Fig. 6, while the relevant pharmacokinetic data are listed in Table 3. The AUC_{rel} values in the table show a remarkable bioavailability increase produced by the inserts compared with the commercial eye-drops. The causes of such an increase have already been discussed in previous papers (Di Colo et al., 2001a,b). CH–HCl produced no substantial bioavailability change with respect to plain PEO 900 or PEO 400, indeed, the AUC_{eff} values for the three insert types are quite similar. However, with the PEO 900–CH–HCl (9:1) insert the C_{max} value was significantly increased over that produced by

Table 2
Behaviour of inserts in the precorneal area: time for complete gelation (TG), residence time (TR) and biocompatibility

Insert material	TG (min)	TR (min)	Irritation signs
PEO 900–CH–HCl (9:1)	30	300	Slight reddening of conjunctiva and eyelid rim
PEO 400[a]	30	180	Slight reddening of conjunctiva
PEO 900[a]	60	360	Slight reddening of conjunctiva and eyelid rim

[a] Data from Di Colo et al. (2001b).

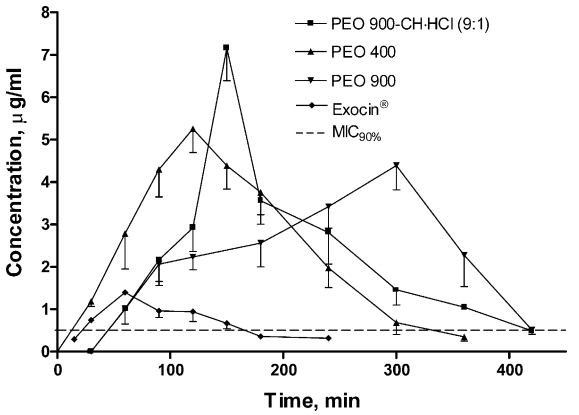

Fig. 6. Profiles of OFX concentration in the aqueous humour of rabbits, following topical administration of 0.3 mg drug by different vehicles. Each data point is the mean ± S.E. of at least six values obtained with different animals. The data for PEO 400, PEO 900 and Exocin® eyedrops have been reproduced for comparison from Di Colo et al. (2001b).

either CH–HCl-free insert. The C_{max} increase with respect to the value for the PEO 900 insert might be explained by an acceleration of drug release caused by CH–HCl, in agreement with the concurrent reduction of t_{max} seen in Table 3 and Fig. 6, and the in vitro release data in Fig. 3 and Table 1. However, the C_{max} increase over the value for the PEO 400 insert can hardly be explained by a faster release with the PEO 900–CH–HCl (9:1) insert, considering that t_{max} is longer for the latter, and that the t_{50} data for the two inserts, seen in Table 1, are similar. More likely, then, the in vivo release was slower with the PEO 900–CH–HCl (9:1) compared with the PEO 400 insert, but CH–HCl, once in contact with the cornea, could exert a gradual enhancing effect on corneal permeability which was responsible for the higher concentration peak in the aqueous.

4. Conclusions

The addition of CH–HCl microparticles to PEO-based inserts produced microstructural changes which accelerated both insert erosion and OFX release. The latter was accelerated more than the former, meaning that CH–HCl increased the diffusive contribution to the release mechanism with respect to the inserts based on plain PEO. The increased release rates, nevertheless, did not produce any major decrease of drug bioavailability, as might be expected from the increased elimination rate from the precorneal area by tear fluid drainage. In fact, dispersion of 10% CH–HCl microparticles in a PEO 900-based insert increased the peak concentration of OFX in the aqueous well over the peak produced by each of the CH–HCl-free PEO inserts, suggesting an ability of CH–HCl to enhance the corneal permeability. Such a property of CH–HCl may be exploited to increase the efficacy of OFX-medicated PEO-based inserts for the topical treatment of endophthalmitis. The results obtained in this work encourage further study aimed at assessing the ability of chemically modified chitosans to enhance corneal permeability, in order to evidence the molecular features of polymers that optimise transcorneal absorption of drugs.

Table 3
Pharmacokinetic parameters for transcorneal penetration into aqueous humour after administration of 0.3 mg OFX in commercial eyedrops (Exocin®), or in ocular inserts based on PEO 900–CH–HCl (9:1), PEO 400 or PEO 900

Vehicle	C_{max} ± S.E. (μg ml^{-1})	t_{max} (min)	AUC_{eff}[a] (μg ml^{-1} min)	AUC_{rel}
Exocin®[b]	1.39 ± 0.05	60	62.3	1
PEO 900–CH–HCl (9:1)	7.16 ± 0.77	150	696.5	11.18
PEO 400[b]	5.25 ± 0.56	120	693.9	11.14
PEO 900[b]	4.39 ± 0.58	300	774.8	12.44

[a] Area under the concentration in the aqueous humour versus time curve and over the $MIC_{90\%}$ level.
[b] Data from Di Colo et al. (2001b).

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