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Relevance of polymer molecular weight to the in vitro/in vivo performances of ocular inserts based on poly(ethylene oxide)

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Abstract

A previous study of the present authors on gel-forming erodible inserts, based on high molecular weight (MW, 400 kDa) poly(ethylene oxide) (PEO), for ocular controlled delivery of ofloxacin (OFX) has been extended to investigate the effects of PEO MW, in the 200–2000 kDa range, on insert properties relevant to therapeutic efficacy. Mucoadhesion has shown a dependence on MW, with a maximum for PEO 400. The in vitro drug release from inserts based on PEO 200, PEO 400 and PEO 900 was mainly controlled by insert erosion, whereas with PEO 2000 it was mainly diffusion-controlled in a first phase, followed by an erosion-controlled phase. The erosion time scale depended directly on MW. Immediately after application in the lower conjunctival sac of the rabbit eye, the inserts based on PEO of whichever MW formed mucoadhesive gels, well tolerated by the animals; then the gels spread over the corneal surface and eroded. PEO 2000 was unsuitable as an insert material, since the resulting gel spilled from the eye, due to excessive swelling. The gel residence time in the precorneal area, the drug permanence time in the aqueous humor at concentrations $>$MIC and the time to reach the maximal drug concentration in the aqueous humor (C_{max}) depended directly on MW, indicating that transcorneal absorption was governed by gel erosion. All inserts increased C_{max} and AUC_{eff} (AUC for concentrations $>$MIC) with respect to the commercial eyedrops. The increases caused by PEO 400 and PEO 900 were similar (3.78- and 3.16-fold, respectively, for C_{max}; 11.06- and 12.37-fold, respectively, for AUC_{eff}), whereas smaller increases were produced by PEO 200. The PEO 400 and PEO 900 inserts have shown a potential for a topical treatment of endophthalmitis.

Keywords: Ocular insert; Ocular drug delivery; Ocular bioavailability; Ofloxacin; Controlled drug delivery; Poly(ethylene oxide); Mucoadhesive polymer

1. Introduction

High molecular weight (10^2–8×10^3 kDa) linear poly(ethylene oxide) (PEO) has shown a great

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potential as a material for controlled drug delivery systems. Matrix tablets based on PEO can be manufactured readily, thanks to the good compressibility of this polymer (Yang et al., 1996). The polyether chains of PEO can form strong hydrogen bonds with water; therefore, when solid matrices are brought into contact with an aqueous medium, the polymer tends to hydrate, forming a superficial gel which eventually erodes as the polymer dissolves. Drug release from such matrices may be controlled by polymer swelling or erosion, or drug diffusion in the hydrated gel, or by these processes altogether. Hence, a variety of release patterns can be obtained, depending on the PEO molecular mass and the drug physicochemical properties. Several studies on PEO-based controlled-release matrices for oral application have been reported (Apicella et al., 1993; Cappello et al., 1994; Kim, 1995; Moroni and Ghebre-Selassie, 1995; Yang et al., 1996; Kim, 1998). Also, applications of PEO as a carrier or component of oral, mucosal and transdermal drug delivery systems are documented by numerous patents (Union Carbide Corp., 1997). Good mucoadhesive properties (Bottenberg et al., 1991) and lack of irritancy to the rabbit eye (Union Carbide Corp., 1996) point to this polymer as an interesting candidate material for controlled-release erodible ocular inserts. Indeed, the research and development of new ocular polymeric ophthalmic drug-delivery systems is desirable, as they show promise of improving drug bioavailability and decreasing side effects, with respect to conventional eyedrops. In a recent paper, Di Colo et al. (2001) described the behavior of an insert based on PEO 400 (molecular mass, 400 kDa), for the intraocular delivery of the potent broad-spectrum fluoroquinolone antibiotic ofloxacin (OFX). The more important features of the insert were: (i) drug release controlled by insert erosion; (ii) fair biocompatibility in the rabbit eye; (iii) attainment of drug levels in the aqueous humor of the rabbit eye above the MIC for the more resistant ocular pathogens; and (iv) an about 10-fold increase of drug bioavailability in the aqueous humor with respect to the commercial OFX eyedrops, commonly used to treat external ocular bacterial infections, such as conjunctivitis and keratitis. The performance of the above PEO-based OFX insert has opened a new prospect of topical treatment of endophthalmitis caused by the more resistant ocular pathogens. Since the insert biopharmaceutics can vary depending on the PEO molecular weight (MW), we have extended the study to evaluate the effects of PEO MW, in the 200–2000 kDa range, on properties potentially relevant to the insert therapeutic efficacy, such as: (i) mucoadhesion; (ii) in vitro OFX release and insert erosion kinetics; (iii) biocompatibility in the rabbit eye; (iv) OFX intraocular pharmacokinetics.

2. Materials and methods

2.1. Materials

The following commercially available materials were used as received. Ofloxacin (OFX) (Sigma, St. Louis, MO, USA), Exocin® eyedrops (Allergan), poly(ethylene oxide) (PEO) of different molecular weights, gifted by Union Carbide Italia S.r.l., Milan, Italy: PEO 200 (Polyox® WSR N-80L, MW 200 kDa); PEO400 (Polyox® WSR N-3000, MW 400 kDa); 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. Methods

PEO loading with OFX, preparation of inserts, measurements of OFX release and insert erosion kinetics in vitro, tests of mucoadhesion, 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 the previous paper (Di Colo et al., 2001). An outline of each procedure is given below.

OFX was adsorbed onto the surface of PEO powder by wetting the powder, portionwise, with an OFX solution in absolute ethanol–methanol 2:1 v/v, while mixing with a spatula and letting the solvent evaporate. The nominal OFX load in PEO was 1.5% w/w. The actual load was deter-

mined spectrophotometrically at 286 nm, following dissolution of powders in pH 7.4 phosphate buffer and filtration through a 0.45 μm pore size membrane. The load values, as determined for three batches of each PEO and expressed as weight fractions, were $1.45 \pm 0.11\%$, for PEO 200; $1.56 \pm 0.15\%$, for PEO 400; $1.48 \pm 0.08\%$, for PEO 900; and $1.52 \pm 0.09\%$, for PEO 2000.

Powders were compressed by a hydraulic press into disk-shaped inserts of 6 mm diameter, 0.8–0.9 mm thickness and 20 mg weight. The nominal drug dose in the medicated inserts was 0.3 mg, a dose corresponding to 2×50 μl Exocin® eyedrops.

For the kinetic measurements in vitro, 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. The disk was immersed, with the exposed insert surface in upward position, into a known volume of isotonic pH 7.4 phosphate buffer, thermostated at 37°C and stirred under controlled hydrodynamics. The OFX release kinetics were determined by spectrophotometrically analyzing the receiving buffer for the drug at 286 μm. To determine the insert erosion kinetics, after a pre-established elution time the disk was withdrawn, dried and weighed, and the dissolved insert weight fraction was computed. This procedure was repeated for different elution times. Approximate estimations of insert swelling kinetics (Fiaschi, 2000) showed that only with the faster eroding inserts, i.e. those based on PEO 200, the maximum swelling degree had been achieved before the shortest elution time of the erosion measurements.

The mucoadhesive potential of each PEO was estimated by measuring the work required to detach the unit surface of sample from a substrate of hog gastric mucin, spread uniformly on wet filter paper. The measurements were performed as described by Saettone et al. (1989). The samples were disks obtained by compression of the polymer powder. For testing, each disk was hydrated for a pre-established time, by immersion in artificial tear fluid prepared as described in the previous paper (Di Colo et al., 2001). The force vs. elongation curves were analysed with KaleidaGraph® software (Synergy Software, Reading, PA, USA).

Male, New Zealand albino rabbits of 2.5–3.0 kg were used for the in vivo tests. 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.

For the evaluation of biocompatibility and residence time in the precorneal area, non-medicated inserts containing sodium fluorescein as a tracer were used. One insert of each type was applied in the lower conjunctival sac of each eye of at least two rabbits. Following insertion, all devices formed a superficial gel and adhered to the application site within 5 min. At appropriate time intervals the state of the release systems 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 hyperhemia

Table 1
Behavior of inserts in the precorneal area: time for complete gelation (TG), residence time (TR), biocompatibility

Insert material	TG (min)	TR (min)	Irritation signs
PEO 200	10	120	None
PEO 400	30	180	Slight reddening of conjunctiva
PEO 900	60	360	Slight reddening of conjunctiva and eyelid rim
PEO 2000	120	>420	None

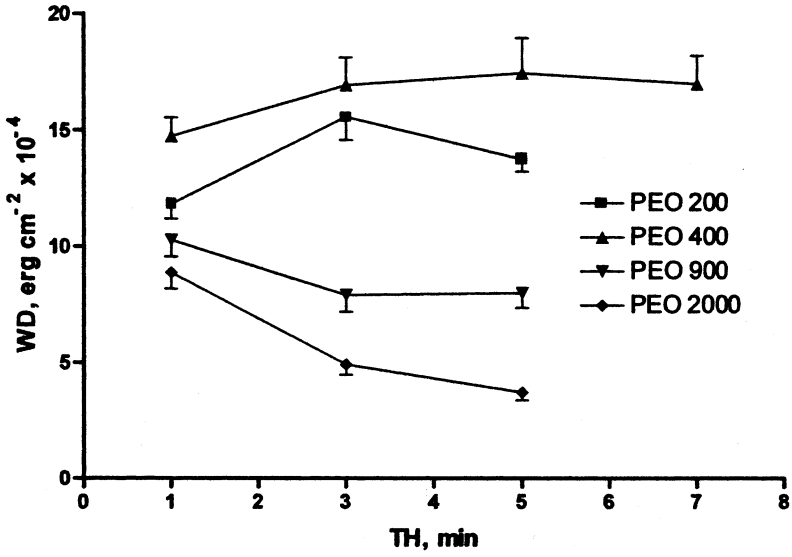

Fig. 1. Results of the mucoadhesion tests. Work of detachment (WD) vs. time of hydration (TH). Each data point is the mean ± S.E. of at least eight measurements. The data for PEO 400 have been reproduced for comparison from Di Colo et al., 2001.

were checked, as well as fluorescence at the rabbit nose, due to lacrimation. Each remark, reported in Table 1, refers to all of the inserts of each type.

For the measurement of OFX transcorneal penetration, each PEO insert, containing a nominal dose of 0.3 mg OFX, or a 100 μl volume (two 50 μl drops, corresponding to 0.3 mg OFX, instilled at a 5 min interval) of the reference Exocin® eyedrops, was applied in the lower conjunctival sac of one eye of each rabbit. After a pre-established time from administration, the rabbits were anaesthetized, then 50–80 μl of aqueous humor were aspirated from the anterior chamber. At least six animals were used for each time point. The aqueous humor 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 were analyzed 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 humor 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. 4), coded AUC_{eff}, was calculated by means of the linear trapezoidal rule (Kaleidagraph, Synergy Software). Difference significance was evaluated by the Student's *t*-test.

3. Results and discussion

3.1. Mucoadhesive potential of PEOs

According to Lejoeux et al. (1988), when the bioadhesive power of different solid or viscous systems is put to comparison, the work required to detach the bioadhesive system from the substrate, equivalent to the area under the force/elongation curve, is a more sensitive parameter than the detachment force. Following this line, we determined the dependence of the detachment work

(DW) on the hydration time (TH) for PEOs of different MW, in order to determine and compare the maximum DW value (DW_{max}) for each MW. As shown in Fig. 1, in all cases such a value was attained within 5 min of hydration. The hydration time corresponding to DW_{max} depended on the polymer MW and was shorter for higher MWs. Indeed, it has been reported that for a given bioadhesive polymer there exists a hydration degree corresponding to an optimum polymer concentration in the gel allowing the maximal interaction with the substrate. Higher concentrations favor polymer–polymer interactions, while with lower concentrations the number of penetrating polymer chains per unit mucous volume is decreased (Bremecker, 1983). The DW_{max} values, plotted in Fig. 2 vs. MW, indicate a higher mucoadhesive potential for the PEOs of lower MW, with a maximum for PEO 400. These results agree with the findings of Bottenberg et al. (1991) who measured the force and energy required to detach buccal tablets prepared with PEOs in the 200–5000 kDa MW range from the mucous membrane of porcine gingiva. In the light of the above evidence, a stronger adhesion of the inserts based on the lower MW PEOs to the corneal epithelium is expected.

3.2. Kinetic measurements in vitro

To gain information on the drug release mechanism from inserts, the kinetics of drug release and insert erosion were measured in vitro, for each of the PEOs under study, and compared. It is seen in Fig. 3 that the release and erosion data relative to PEO 200 are not significantly different, thus pointing to a completely erosion-controlled release in this case. Accordingly, the relevant release kinetics are of zero-order up to around 75% dose released. On the other hand, with PEO 2000 the release rate significantly exceeds the erosion rate during the first 3 h of experiment, thus indicating an essentially diffusion-controlled mechanism in this phase, followed by a period where the two rates tend to become equal and erosion takes control of the release process. The prevailing contribution of diffusion in the initial phase of release is also indicated by a burst effect, which is particularly evident with PEO 2000. It appears from Fig. 3 that the erosion time scale is related directly, and therefore, the erosion rate is related inversely, to the polymer MW. A similar relationship between erosion rate and MW was reported for hydroxypropyl methylcellulose by Reynolds et al. (1998). The authors explained such a relation-

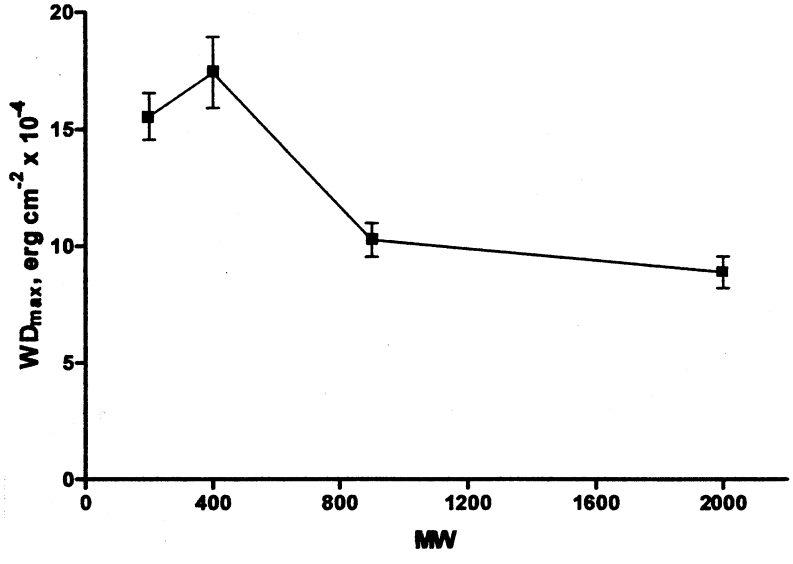

Fig. 2. Results of the mucoadhesion tests. Maximum work of detachment (WD_{max}) as a function of polymer MW.

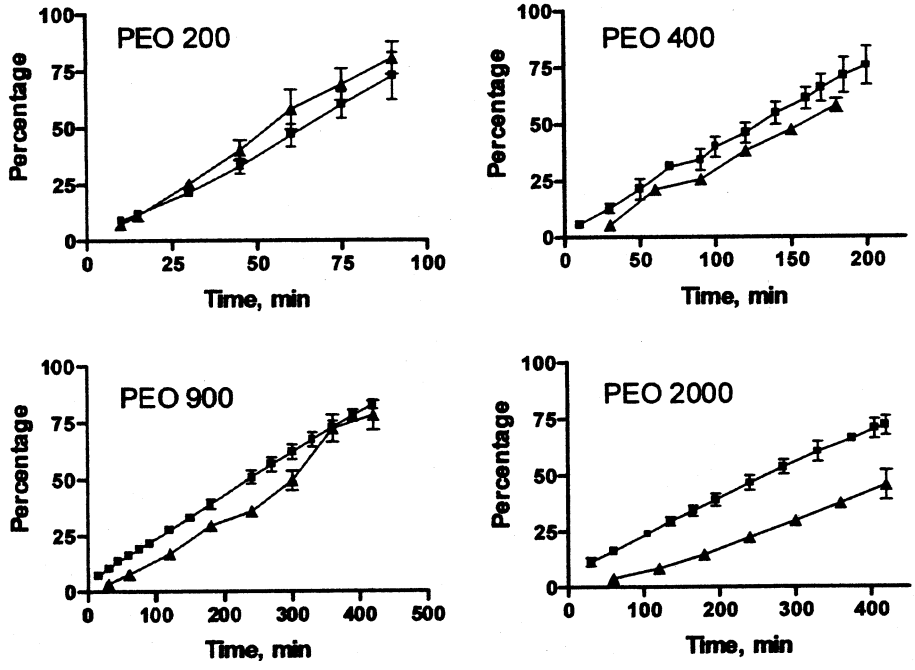

Fig. 3. In vitro drug release and insert erosion kinetics for inserts based on PEO of different MW, medicated with 1.5% (0.3 mg) OFX. Key: ■, dose released; ▲, insert eroded. Each data point is the mean ± S.D. of at least three values. The data for PEO 400 have been reproduced for comparison from Di Colo et al. (2001).

ship by arguing that polymer dissolution was mass transfer limited, that the critical polymer concentration in the gel eliciting chain disentanglement established the driving force for mass transfer, and that the disentanglement concentration of polymer followed an inverse relationship with MW. This theory is believed to apply as well to the erosion of the present PEOs. Since drug diffusion and polymer erosion are parallel drug release mechanisms, the faster of the two would be controlling the release process. Indeed, the present data show that release was the more erosion-controlled the lower was the PEO's MW.

3.3. Animal tests

3.3.1. Evaluation of biocompatibility and residence time of inserts in the precorneal area

The behavior of non-medicated inserts based on the different PEOs in rabbit eyes is described in Table 1. In all cases, the inserts adhered almost instantly to the application site, as they formed gels that gradually spread over the corneal surface and finally eroded. From the remarks reported in Table 1 it appears that these phenomena were faster with the lower MWs, in agreement with the erosion behavior of PEOs in vitro. As a consequence of the comparatively low erosion rate of PEO 2000, this insert swelled so much that the resulting gel partly spilled from the eye. Therefore, this PEO was not used for the following pharmacokinetic studies. None of the inserts caused important irritation signs. Some slight reddening of the eyelid rim and/or the conjunctiva, not apparently related to the PEO MW, was noticed in some instances.

3.3.2. OFX transcorneal penetration

The OFX concentration profiles in the aqueous humor, following administration of a 0.3 mg dose by the systems under study are compared in Fig. 4, while the relevant pharmacokinetic data are listed in Table 2. A direct correlation with MW, which was shown by the drug release and matrix

erosion time scale in vitro and by the residence time in the precorneal area, is also shown by the t_{max} and t_{eff} values in Table 2, which indicates that drug absorption into the aqueous humor was largely governed by the erosion-controlled release from the PEO systems. In particular, PEO 900 prolonged the t_{eff} up to about 2.5 times the value for the reference eyedrops. The AUC_{eff} values for the PEO inserts, seen in Table 2, are remarkably higher than the reference value. It is known that the ocular bioavailability of drugs administered as eyedrops is very low, due to a rapid drug clearance from the precorneal area by lacrimal drainage and turnover. With the inserts, on the other hand, the viscosity of the tear fluid was increased by the dissolved PEO, which stabilized such a fluid and reduced the drug elimination rate from the precorneal area, thereby increasing the bioavailability. However, the AUC_{eff} value for the PEO 200 insert is significantly lower than the values for PEO 400 and PEO 900, which indicates that PEO 200 allowed absorption of a lower dose fraction, probably because dissolution of this PEO in the tear fluid produced a comparatively low viscosity increase, and hence, a comparatively low decrease of the elimination rate from the precorneal area. Indeed, the viscosity values of 5% aqueous solutions of PEO 200, PEO 400 and PEO 900, as reported by the manufacturer, are 65–90, 2250–4500 and 8800–17600 cP, respectively (Union Carbide Corp., 1993). The faster precorneal clearance for PEO 200 relative to PEO 400 also resulted in a significantly lower C_{max} for the former ($P < 0.001$), in spite of its higher drug release rate. The AUC_{eff} values for PEO 400 and PEO 900, seen in Table 2, are similar, in fact, the difference between them is well within the relevant data variability, apparent in Fig. 4. Such a similarity, implying a similar dose fraction penetrated into the aqueous in the two cases, prompts the following consideration: either the above mentioned difference in viscosity increasing power between these two PEOs was not sufficient to produce a significant difference in precorneal clearance, or a faster precorneal clearance in the case of the less viscous PEO 400 was compensated for by a higher corneal permeability induced by this PEO. In favor of the latter hypothesis are some data of the previous article (Di Colo et al., 2001), which were interpreted by admitting an increase of corneal permeability, due to intimate contact with the more bioadhesive PEO 400 vehi-

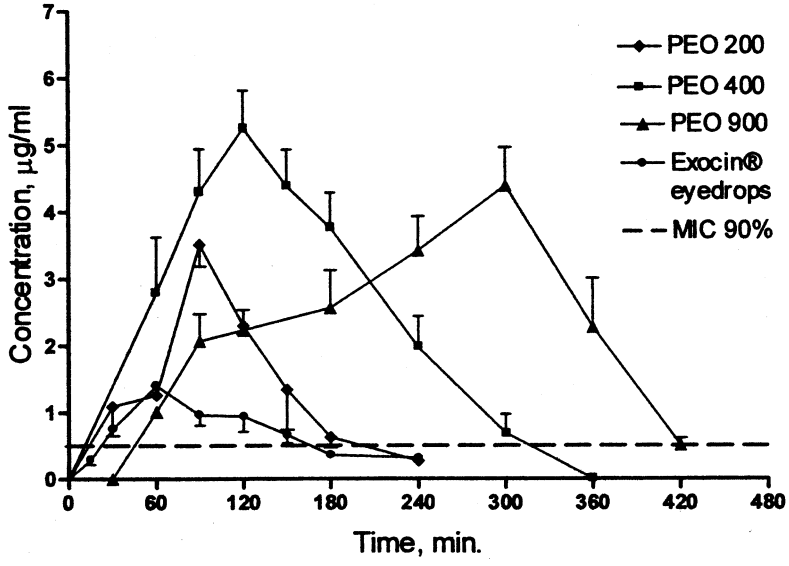

Fig. 4. Profiles of OFX concentration in the aqueous humor of rabbits, following topical administration of 0.3 mg OFX by different vehicles. Each data point is the mean ± S.E. of at least six values obtained with different animals. The data for PEO 400 and Exocin® eyedrops have been reproduced for comparison from Di Colo et al. (2001).

Table 2
Pharmacokinetic parameters for transcorneal penetration into aqueous humor after ocular administration of 0.3 mg OFX in commercial eyedrops (Exocin®), or in ocular inserts based on PEO 200, PEO 400, or PEO 900

Vehicle	C_{max}[a] ± S.E. (μg/ml)	t_{max}[b] (min)	AUC_{eff}[c] (μg/ml min)	t_{eff}[d] (min)	AUC_{rel}[e]
Exocin®[f]	1.39 ± 0.05	60	62.75	148	1
PEO 200	3.51 ± 0.32	90	208.13	195	3.32
PEO 400[f]	5.25 ± 0.56	120	693.61	290	11.06
PEO 900	4.39 ± 0.58	300	775.53	380	12.37

[a] Maximal OFX concentration in the aqueous humor.
[b] Time to reach C_{max}.
[c] Area under the concentration in the aqueous humor vs. time curve and over the $MIC_{90\%}$ level (see Fig. 4).
[d] Time of permanence of the concentration in the aqueous humor at values $> MIC_{90\%}$.
[e] Ratio of AUC_{eff} to the value for the reference Exocin®.
[f] Data taken for comparison from Di Colo et al., 2001.

cle. The present findings, although insufficient to prove such a hypothesis, agree with it, so far as the mucoadhesion data of Fig. 2 show a higher mucoadhesive potential of PEO 400 compared to PEO 900.

4. Conclusions

Correlations between PEO MW and insert properties potentially related to its therapeutic efficacy have been evidenced. On this basis, PEO 400 and PEO 900 have been recognized as the more promising members of the PEO polymer class. With these MWs, drug release from inserts and transcorneal absorption was essentially controlled by polymer erosion, therefore, the slower eroding PEO 900 ensured effective antibiotic levels in the aqueous for a time substantially longer than the commercial eyedrops, with a potential to reduce the administration frequency. PEO 400, which eroded faster than PEO 900, produced a concentration peak in the aqueous humor significantly higher ($P < 0.002$) than 4 μg/ml, that is, the $MIC_{90\%}$ for the more resistant ocular pathogens (Taravella et al., 1999). Either PEO 400 or PEO 900 showed the additional, remarkable advantage of increasing the OFX effective availability in the aqueous humor by one order of magnitude with respect to the commercial eyedrops. The lower MW PEO 200 allowed lower levels and bioavailability of the antibiotic in the aqueous humor, probably because of its limited ability to increase the tear fluid viscosity. The higher MW PEO 2000, on the other hand, was unsuitable as it partly spilled from the rabbit eye, because of an excessive swelling, due to a comparatively low erosion rate. The question, raised in the previous report (Di Colo et al., 2001), about the PEO's ability to increase the corneal permeability by virtue of its mucoadhesive power still remains unresolved, since a clear direct correlation between PEO mucoadhesion potential and OFX bioavailability could not be established. Indeed, the latter was also influenced directly by the PEO ability to increase the tear fluid viscosity and the two PEO properties were correlated with the polymer MW in opposite ways. A fair biocompatibility of the PEO systems in the rabbit eye has been observed, whichever the PEO's MW. In conclusion, the present inserts show promise of considerable therapeutic advantages, the prominent of which is the possibility of an efficacious topical treatment of endophthalmitis.

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