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Research paper

Silicone rubber/hydrogel composite ophthalmic inserts: preparation and preliminary in vitro/in vivo evaluation

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Abstract

The present report describes the development and in vitro/in vivo testing of rod-shaped mucoadhesive ophthalmic inserts fitting the upper or lower conjunctival fornix. Cylindrical devices (diameter 0.9 mm, length 6–12 mm, weight 3–8 mg) all containing 0.8 mg oxytetracycline HCl (OXT) were prepared from appropriate mixtures of silicone elastomer, OXT and sodium chloride as release modifier. A stable polyacrylic acid (PAA) or polymethacrylic acid (PMA) interpenetrating polymer network (IPN; 30 or 46% w/w) was grafted onto the inserts' surface by treatment with a mixture of acrylic (or methacrylic) acid and ethylene glycol dimethacrylate in xylene at 100°C. Mucoadhesion studies in vitro showed that the mucoadhesive properties increased significantly with increasing thickness of the IPN layer. The inserts were tested for drug release in vitro, and for drug release and retention in rabbit eyes. The presence of IPN, as well as of NaCl, in general increased the drug release rate. The PMA-grafted devices released OXT at lower rates when compared with the PAA-grafted ones. A nearly zero-order release rate for about 1 week was observed in vitro for some types of inserts. When tested in rabbits, some IPN-grafted inserts maintained in the lacrimal fluid a OXT concentration of 20–30 μg/ml for several days: the in vitro minimum inhibitory concentration values (MIC 90%) of OXT against micro-organisms responsible of common ocular infections range from 0.8 to 2.0 μg/ml, while MIC 90% values in the range 14-50 μg/ml have been indicated for *Pseudomonas aeruginosa*. The ocular retention of IPN-grafted samples was significantly higher with respect to ungrafted ones. The presently described mucoadhesive silicone inserts might prove efficient therapeutic systems for chemotherapy of ocular bacterial infections, such as trachoma.

Keywords: Ocular inserts; Silicone; Polyacrylic acid; Polymethacrylic acid; Mucoadhesive ocular inserts; Oxytetracycline; In vitro mucoadhesion; In vitro/in vivo drug release; Ocular retention; Rabbit studies

1. Introduction

In spite of intensive research and promotion, solid ocular delivery devices (inserts) have never gained the wide popularity and acceptance enjoyed by traditional liquid and semi-solid formulations (eyedrops, ointments and hydrogels). Ophthalmic inserts, however, are claimed to possess distinct advantages over standard formulations [1]. Their prolonged retention in the conjunctival sac can significantly increase the topical bioavailability of ophthalmic drugs. Furthermore, if properly engineered, inserts can release drugs at sustained and/or controlled rate, thus providing improved therapeutic efficacy and a lower incidence of side-effects. As a further benefit, solid delivery systems can potentially increase the safety of topical therapy by significantly reducing systemic drug absorption.

A number of investigations on inserts have been devoted to the critical issues of prolonged retention and constant-rate drug delivery. The cylindrical, rod-shape has been declared

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by different authors to provide optimal retentive properties [2–4]. Zero-order drug delivery for over 2 weeks can be realized by a variety of approaches, in some cases simpler than that epitomized by the now classical Ocusert®, a complex membrane/reservoir device [5]. In particular, the Ocufit SR®, a rod-shaped insert made of silicone elastomer, fitting the shape and size of the human conjunctival fornix and patented in 1992, was claimed to combine long retention and sustained release properties [6].

Preliminary tests on rod-shaped silicone ocular inserts manufactured in our laboratory indicated for these devices a relatively high rate of expulsion from rabbit eyes during long-term treatment. This effect was attributed to the lack of adhesive interactions between silicone rubber (hydrophobic) and the palpebral and scleral mucosae (hydrophilic). It was therefore attempted to improve the ocular retention of the inserts by grafting onto their surface hydrophilic, mucoadhesive polyacrylic or polymethacrylic acid layer. Hydrophilicity gradients were introduced into the inserts' silicone network by allowing acrylic or methacrylic acid to diffuse into it and simultaneously polymerize, thus forming a gradient interpenetrating polymer network (IPN) layer. Theoretical and experimental details of the procedure have been described in the literature [7,8].

The preparation and in vitro/in vivo testing of hydrogel-grafted silicone inserts releasing oxytetracycline constitutes the object of the present report. The tetracyclines are 'broad-spectrum' antibacterial drugs, active against many common gram-positive and gram-negative bacteria, rickettsiae, etc., causing ocular surface infections such as conjunctivitis and keratitis, as well as against less common pathogen agents as *chlamydia trachomatis* and *neisseria gonorrhoeae*.

2. Materials and methods

2.1. Materials

Oxytetracycline HCl (OXT) was kindly given by I.M.S., s.r.l. (Milano, Italy); medical grade polydimethylsiloxane elastomer (PDMS; Silastic® MDX-4-4210) and curing agent (CA) were purchased from Dow Corning, Midland, MI, USA; ethylene glycol dimethacrylate (EGDMA), α,α'-azoisobutyronitrile (AIBN), acrylic acid (AA) and methacrylic acid (MA) were obtained from Fluka AG, Buchs, Switzerland. Sodium chloride (NaCl; Carlo Erba, Milano, Italy) was sieved to a 20–40 μm size range [9]. Hog gastric mucin (HGM) was purchased from Tokyo Kasei Kogyo, Tokyo, Japan.

Buffer substances and all other chemicals or solvents were of reagent grade.

2.2. Preparation of inserts

Polydimethylsiloxane rod-shaped silicone inserts (RSI) were prepared using appropriate amounts of PDMS, CA, OXT and NaCl (as release modifier). The mixtures, whose composition is reported in Table 1, were injected into aluminium moulds (diameter 0.9 mm, length 22.0 mm), and were allowed to cure at 45°C for 24 h.

The resulting rubbery cylinders (diameter 0.9 mm, length 22 mm) were appropriately cut to give a OXT content of 0.8 mg. The final lengths and weights were in the range 4–12 mm and 2.7–8.0 mg, depending on insert type (See Table 2). The RSI were used, as such and after polyacrylic acid (PAA) or polymethacrylic acid (PMA) coating, for hydration tests and for in vitro/in vivo drug release studies.

Flat, disk-shaped inserts (DSI) for in vitro mucoadhesion tests were prepared by accurately spreading the same 10:1 mixture of PDMS and CA on a Teflon® mould (diameter 50 mm). After curing at 45°C for 24 h, the resulting rubbery films were cut in the shape of disks (average thickness 1.0 mm, diameter 12.0 mm). These were used for the tests after PAA or PMA surface-grafting.

2.3. Inserts hydrogel-grafting procedure

Grafting of a PAA or PMA IPN gradient onto PDMS cylindrical and disk-shaped inserts (RSI and DSI) was essentially carried out by the method described in a previous paper [8].

In brief, the devices (5-10 RSI or DSI) were swollen in boiling xylene (10.0 ml), then were introduced into a flask containing 7.7 ml xylene, 0.22 ml ethanol, 2.3 ml AA (or 2.8 ml MA, both freshly distilled in vacuo) and 64.5 mg EGDMA. A small amount (5.0 mg) of AIBN initiator dissolved in xylene (1.0 ml) was quickly added, and the mixture was refluxed for 1 h. A nest of glass wool prevented the contact of the devices with the flask walls. After completion of the polymerization reaction, the inserts were recovered from the spongy mass of PAA or PMA, briefly washed with ethanol and dried in vacuo at 100°C for 12 h. This procedure ensured complete removal of xylene and of non-reacted materials.

Some once-grafted inserts were submitted again to the above procedure to increase the thickness of IPN layer.

The reproducibility of the procedure was quite satisfactory: the percent w/w PAA or PMA content of the grafted inserts was 30.0 ± 0.71 and 46.0 ± 0.95 after one or two polymerization reactions, respectively.

Table 1

Composition of the mixtures used for the preparation of rod-shaped inserts (RSI)

Insert type	PDMS + CA 10:1 (% w/w)	OXT (%w/w)	NaCl (%w/w)
RSI-10	70.0	10.0	20.0
RSI-15a	85.0	15.0	–
RSI-15b	70.0	15.0	15.0
RSI-30	70.0	30.0	–

Table 2

Characteristics of rod-shaped inserts (RSI)

Insert type	Diameter (mm)	Weight (mg)/ length (mm)	NaCl (%)	Grafting
RSI-10	0.9	8.0/12.0	20.0	None
RSI-15a	0.9	5.3/8.0	–	None
RSI-15b	0.9	5.3/8.0	15.0	None
RSI-30	0.9	2.7/4.0	–	None
RSI-10/PAA1	1.0	11.4/13.5	20.0	PAA, single grafting
RSI-15a/PAA1	1.0	7.6/9.0	–	PAA, single grafting
RSI-15b/PAA1	1.0	7.6/9.0	15.0	PAA, single grafting
RSI-30/PAA1	1.0	3.8/4.5	–	PAA, single grafting
RSI-10/PAA2	1.2	14.8/13.8	20.0	PAA, double grafting
RSI-15a/PAA2	1.2	9.9/9.2	–	PAA, double grafting
RSI-15b/PAA2	1.2	9.9/9.2	15.0	PAA, double grafting
RSI-30/PAA2	1.2	4.9/4.6	–	PAA, double grafting
RSI-15a/PMA1	1.0	7.6/9.0	–	PMA, single grafting
RSI-30/PMA1	1.0	3.8/4.5	–	PMA, single grafting
RSI-15a/PMA2	1.2	11.4/9.2	–	PMA, double grafting
RSI-30/PMA2	1.2	4.9/4.6	–	PMA, double grafting

2.4. Hydration tests

Hydration studies on once or twice PAA- (or PMA-) grafted RSIs were performed by maintaining the inserts in 66.7 mM pH 7.38 Sörensen phosphate buffer at 30°C. At appropriate intervals, the inserts were withdrawn from the solution, superficially dried and weighed. The hydration was calculated as percent weight increase and plotted vs. time.

2.5. Mucoadhesion tests

The mucoadhesive properties of PAA- and PMA-grafted DSI were evaluated by measuring their work of adhesion (W) on a mucin substrate [10]. The latter consisted of a 25% w/w dispersion of HGM in water, spread uniformly on wet filter-paper [11]. The apparatus consisted of a testing cell connected to a custom-made tensile apparatus fitted with force and elongation transducers, whose output was fed to a computer equipped with data acquisition software (TP 5008, TiePie Engineering, Leeuwarden, The Netherlands). For testing, the DSIs were placed between the upper and lower mucous surface of the testing cell in the absence of external bathing fluid; the overall applied load (upper cell weight) was 7.8 g. The detachment tests were performed after 20 min of contact, at 30 ± 0.5°C. The resulting force vs. elongation curves were analyzed using the Kaleida-Graph® software (Synergy Software, Reading, PA, USA). The reported W values, corresponding to the areas under the curves, are the average of at least six determinations.

2.6. In vitro release of oxytetracycline HCl (OXT)

In vitro release tests were carried out on all medicated RSIs. For these studies, glass vials containing one insert in 10.0 ml of pH 7.4, 66.7 mM phosphate buffer were placed in a thermostated (30 ± 0.5°C) shaking water bath. At appropriate intervals the solution was completely withdrawn for high performance liquid chromatography (HPLC) analysis, and replaced with fresh buffer. Sampling was discontinued after 2 weeks.

2.7. Animal studies: oxytetracycline HCl (OXT) release to tear fluid of rabbits

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). The animals were housed in standard cages in a light-controlled room at 19 ± 1°C and 50 ± 5% relative humidity, 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. All experiments were carried out under veterinary supervision, and the protocols were approved by the ethical-scientific committee of the University.

The studies of OXT release to the lacrimal fluid were performed as follows. One RSI (once- or twice-coated) was introduced into the lower conjunctival sac of one eye of the rabbit; the eyelids were then gently kept closed for 30 s. At different times after administration (10, 30, 60, 120 min, 4.5 and 7 h) tear fluid samples were collected from the lower marginal tear strip as described by Urtti et al. [12], using 1.0 μl disposable glass capillaries (Drummond 'Microcaps', Fisher Scientific, St. Louis MO, USA). The samples were transferred into microtubes, and the capillaries were flushed several times with distilled water. After dilution to 100 μl with distilled water, the samples were stored at −18°C before HPLC analysis. At least six eyes were used for each time point.

2.8. Analytical method

OXT analysis was carried out by HPLC. The apparatus (Shimadzu, Kyoto, Japan) consisted of LC 10AS pump, 20 μl Rheodyne injector, SPD-10A UV detector and C-R4A integrating system. The column (Bondclone 30 × 3.9 mm; Phenomenex, Torrance, CA, USA) was packed with μ-Bondpack® C18 (pore size 10 μm) and fitted with a pre-column (Guard-Pack® Holder, Waters, Milford, MA, USA). The mobile phase (flow rate 1.3 ml/min) was methanol/water (40:60) containing 1% p/v triethylamine, adjusted to pH 3.0 with phosphoric acid. Detection was performed at 361 nm; the OXT retention time was 4.3 min. The limit of quantitation (LOQ) of the method was 95 ng/ml.

3. Results and discussion

3.1. Characteristics of rod-shaped inserts (RSI)

The characteristics of the different types of ungrafted and hydrogel-grafted RSI, all containing 0.8 mg OXT, are illustrated in Table 2.

3.2. Hydration studies

The results of hydration tests carried out on four differently hydrogel-grafted RSIs are reported in Fig. 1 as percent water absorbed vs. time. The inserts submitted to these tests were RSI-15a/PAA1, RSI-15a/PAA2, RSI-15a/PMA1 and RSI-15a/PMA2 (once- and twice-grafted with PAA and PMA, respectively). After 8 h (approximately at equilibrium) the weight increases for the once and twice PAA-grafted inserts were 45.0 and 70.0% w/w, respectively, while the corresponding once and twice PMA-grafted inserts showed lower weight increases (15.0 and 34.0% w/w, respectively). Thus, the data indicate an increase of the hydration degree, and hence of the hydrophilic character, with increasing thickness of the IPN layer. The type of grafted IPN layer appeared also to exert a significant effect on hydration: the more hydrophilic PAA-grafted layer(s) favored absorption of greater amounts of water when compared with the less hydrophilic PMA ones.

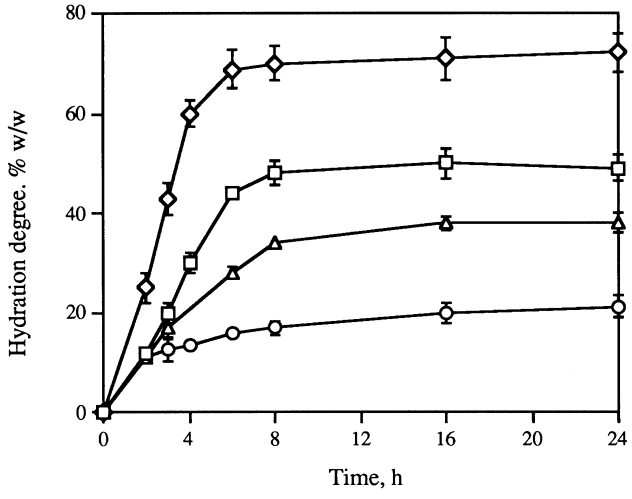

Fig. 1. Hydration vs. time profiles of rod-shaped inserts (RSI). (○), RSI-15a/PMA1; (△), RSI-15a/PMA2; (□), RSI-15a/PAA1; (◇), RSI-15a/PAA2. Vertical bars represent SE ($n = 4$).

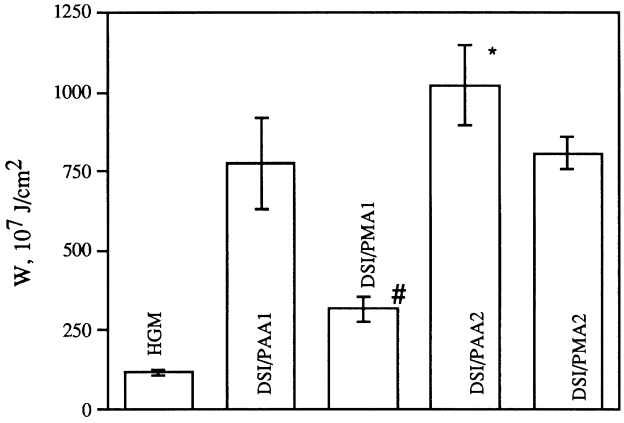

Fig. 2. Work of adhesion (W) of differently grafted disk-shaped inserts (DSI) on a mucin substrate. The first bar (HGM) indicates the work of self-adhesion of the substrate. Vertical bars represent SE ($n = 6$). *Significantly different from DSI/PAA1 ($P < 0.05$, Fisher PLSD test). #Significantly different from all DSI ($P < 0.05$, Fisher PLSD test).

3.3. Mucoadhesion tests

The results of the mucoadhesion tests, carried out on DSI, are reported in Fig. 2 as the work (W; J/cm^2) required for detachment of two hydrated mucin surfaces between which was placed one insert.

The first bar in the graph (HGM) indicates the W value of the two mucin surfaces in absence of interposed insert, and represents the work of cohesion of hydrated mucin.

The W values for DSI/PAA2 and DSI/PMA2 were in the range 8.0–10.0 10^{-5} J/cm^2, close to 10.75 10^{-5} J/cm^2, the W value obtained with a reference Na polyacrilate DSI (not reported in the graph). Since the mucoadhesive performances of polyacrylate (Carbopol 940) are considered excellent, the data are indicative of the good mucoadhesive properties conferred to silicone inserts by PAA- and PMA-grafting. The mucoadhesive properties of the inserts increased significantly with increasing thickness of their IPN layers: the W values were 7.19 and 10.22 10^{-5} J/cm^2, respectively for DSI/PAA1 and DSI/PAA2, while they were 3.17 and 8.08 10^{-5} J/cm^2, respectively for DSI-PMA1 and DSI/PMA2. Such a dependence of mucoadhesion on thickness of IPN layer is probably related to the different composition gradients of IPN layers of different thickness. Statistically significant differences ($P < 0.05$) were observed among the mucoadhesion values measured for the different DSIs; only the difference observed between DSI/PMA2 and DSI/PAA1 was not statistically significant ($P > 0.05$, ANOVA, followed by group comparison with Fisher PLSD test).

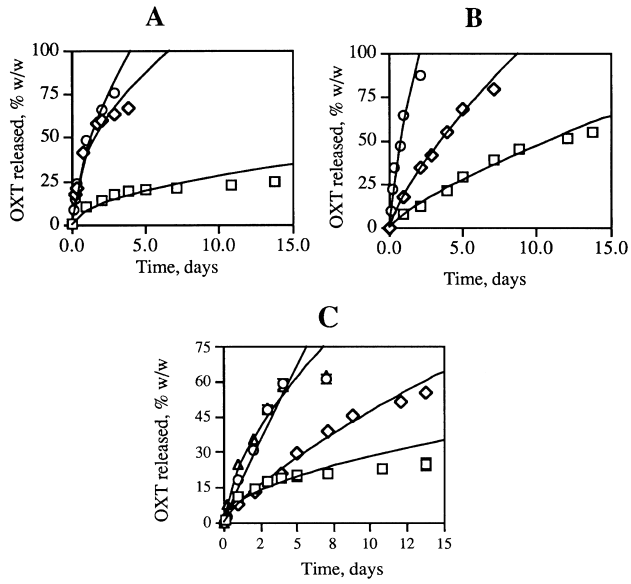

Fig. 3. In vitro release profiles of OXT from different RSI. (A) (□), RSI-15a; (◇) RSI-15a/PAA1; (○) RSI-15a/PAA2. (B) (□), RSI-30; (◇) RSI-30/PAA1; (○) RSI-30/PAA2. (C) (□), RSI-15a; (○) RSI-15a/PMA2; (◇) RSI-30; (△) RSI-30/PMA2. Vertical bars represent SE ($n = 4$). When bars are not present, the SE was smaller than the size of symbol.

3.4. Oxytetracycline HCl (OXT) release in vitro

The in vitro release profiles of OXT from the different rod-shaped inserts are illustrated in Fig. 3. The release kinetics were analyzed using the semiempyrical relationship $M_t/M\infty = Kt^n$, where $M_t/M\infty$ is the fraction of drug released at time t, K is a constant, characteristic of the system and the exponent n is indicative of the release kinetics [13,14]. A value of $n = 0.5$ indicates the occurrence of Fickian diffusion, while $n = 1$ corresponds to zero-order kinetics. Values of n between 0.5 and 1 indicate anomalous (non-Fickian) transport. The main in vitro release parameters are reported in Table 3. Table 3 reports for each insert, in addition to the n and K values, the $t_{20\%}$ and $t_{40\%}$ values (times required for release of 20 and 40% OXT, respectively), and the $R_{20\%}$ and $R_{40\%}$ values (instantaneous release rates at 20 and 40% released drug). Even if some calculated interpolation curves (Fig. 3C) appeared to indicate a non-ideal fit, the relevant correlation coefficients (r) were in the range 0.967–0.996.

The n values of the ungrafted matrices, ranging from 0.610 to 0.824, are indicative of an 'anomalous' release mechanism. Further inspection of the release data shows a dependence of the release rate on the drug content. A comparison, e.g., of inserts RSI-15a and RSI-30, containing 15 and 30% OXT respectively, and no NaCl, indicates a faster release in the case of the latter insert ($R_{20\%}$ = 2.77 and 4.58 days^{-1}, respectively). This effect is probably due to greater porosity induced in RSI-30 by the higher drug loading.

The matrices RSI-15b and RSI-30, containing overall 30% soluble salts (15% OXT + 15% NaCl or 30% OXT, respectively) and presumably having the same porosity, showed higher release rates and larger drug amounts released after 10 h with respect to both matrices RSI-10, having the same overall salts content but containing 20% NaCl, and RSI-15a, containing only 15% OXT and no NaCl. The latter two inserts actually released only 25% of their drug content after 10 days. The small amount of released OXT was presumably due to the poor solubility of the drug in the silicone inserts.

It can be speculated that NaCl in the matrices may exert two opposing actions: (i) a release-promoting effect, due to osmotically-activated formation of water-filled pores; (ii) a release-reducing effect resulting from the presence of the common Cl^- ion. Either effect might prevail depending on the amount (15 or 20%) of NaCl in the matrix.

The presence of a PAA IPN layer in general increased the

Table 3
OXT release parameters in vitro

Insert type	n	K (days^{-n})	$t_{20\%}$ (days)	$R_{20\%}$ (days^{-1})	$t_{40\%}$ (days)	$R_{40\%}$ (days^{-1})
RSI-10[a]	0.690	6.62	4.97	2.76	–	–
RSI-15a	0.610	8.09	4.40	2.77	–	–
RSI-15b[b]	0.824	12.27	1.81	9.11	4.20	7.85
RSI-30	0.780	7.69	3.40	4.58	8.28	3.76
RSI-10/PAA1[a]	0.949	12.08	1.69	11.16	3.55	10.75
RSI-15a/PAA1	0.386	39.26	0.17	44.98	1.05	14.71
RSI-15b/PAA1[b]	0.450	43.11	0.31	29.23	1.42	12.65
RSI-30/PAA1	0.767	18.97	1.07	14.32	2.64	11.60
RSI-10/PAA2[a]	0.389	54.07	0.077	98.45	0.46	33.57
RSI-15a/PAA2	0.612	42.85	0.28	42.50	0.89	27.40
RSI-15b/PAA2[b]	0.482	86.50	0.048	00.0	0.20	94.98
RSI-30/PAA2	0.677	60.38	0.19	69.32	0.54	49.76
RSI-15a/PMA2	0.955	14.19	1.43	13.33	2.95	12.90
RSI-30/PMA2	0.620	22.64	0.81	15.16	2.48	9.93

[a]20% w/w NaCl.
[b]15% w/w NaCl.
$t_{20\%}$ and $t_{40\%}$, times required for release of 20 and 40% OXT, respectively.
$R_{20\%}$ and $R_{40\%}$, instantaneous release rates at 20 and 40% released drug, respectively.

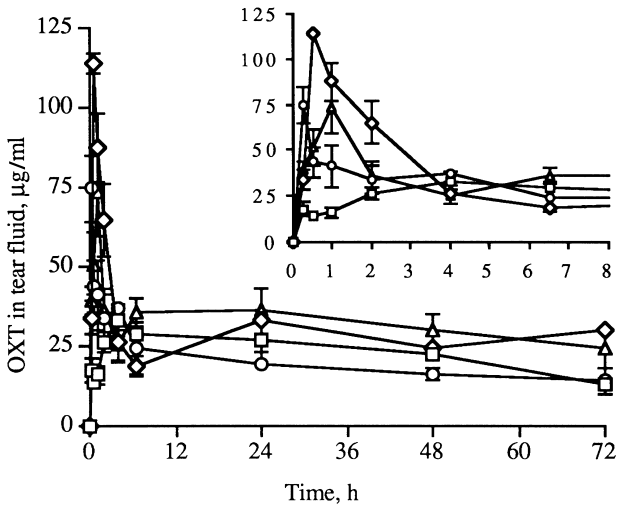

Fig. 4. OXT concentration profiles in tear fluid of rabbits after administration of twice-grafted RSI. (□) RSI-15a/PAA2; (◇) RSI-30/PAA2; (○) RSI-10/PAA2; (△) RSI-15b/PAA2. For clarity, the release profiles during the first 8 h are reported in the inside graph. Vertical bars represent SE (n = 6).

drug release rate, as evidenced by the release profiles in Fig. 3 and by the data in Table 3. For the matrices grafted with a lower amount of PAA (PAA1), the $R_{20\%}$ values and the amount of OXT released after 10 days were substantially higher with respect to the corresponding ungrafted matrices.

In particular, the matrices RSI-10/PAA1, RSI-15b/PAA1 and RSI-30/PAA1 released 80–90% OXT in 12 days, while the matrix RSI-15a/PAA1, containing 15% OXT, released a maximum of 70% OXT after 7 days. The increased OXT release rate resulting from PAA-grafting is presumably due to the increased hydrophilic character induced by the presence of the PAA IPN layer, as also evidenced by the hydration studies.

Preliminary tests on in vitro OXT release from inserts grafted with a lower amount of PMA (PMA1) indicated no significant increase of drug release with respect to analogous ungrafted samples (data not reported). A release study was thus performed only on twice-grafted (PMA2) inserts. The results, reported in Table 3 and Fig. 3, show that the PMA2-grafted devices released OXT at lower rates when compared with the corresponding PAA2-grafted samples, and with analogous rates when compared with the once-grafted PAA1 ones.

Furthermore, PMA-grafting of the matrices containing 15% OXT (RSI-15a/PMA2) produced a change in drug release kinetics from anomalous to near zero-order. The calculated n values were 0.610 and 0.955 for RSI-15a and RSI-15a/PMA2, respectively.

3.5. Oxytetracycline HCl (OXT) release to tear fluid in vivo

Preliminary experiments showed that retention in rabbit eyes of twice-grafted inserts was significantly higher with respect to once-grafted ones, independently of the type of IPN. Retention times longer then 4 days were observed for twice-grafted PMA and PAA inserts in 70 and 40% of the cases, respectively. The reduced occurrence of expulsion from the conjunctival sac of twice-grafted PMA devices with respect to PAA ones was presumably due to their inferior degree of swelling, as confirmed by the hydration profiles (Fig. 1), and consequently to a reduced increase in size.

Therefore, release studies in vivo were carried out only on twice-grafted inserts (RSI-10/PAA2, RSI-15a/PAA2, RSI-15b/PAA2, RSI-30/PAA2, RSI-15a/PMA2 and RSI-30/PMA2). The OXT concentration profiles in tear fluid over a period of 72 and 36 h, resulting from application respectively of PAA- and PMA-grafted matrices, are illustrated in Figs. 4 and 5, while the relevant pharmacokinetic parameters are reported in Table 4. Even if the inserts were retained in the conjunctival sac for periods longer than 4 (PAA-grafting) and 3 days (PMA-grafting), after these times the drug was no longer detectable in tear fluid by the present analytical method.

The in vivo OXT release profiles for all tested matrices exhibited two distinct stages: an initial pulse, lasting 2 and 4 h for PMA- and PAA-grafted inserts, respectively, characterized by a rapid concentration increase up to a maximum value (C_{max}) and followed by rapid decrease, and a second period characterized by a concentration plateau of 68 and 35 h for PAA- and PMA-grafted matrices, respectively. In particular, the inserts containing 30% OXT (RSI-30) produced a higher initial concentration pulse in tear fluid after 30 min with respect to the corresponding inserts containing 15% drug (113.8 and 69.4 μg/ml for the RSI-30/PAA2 and RSI-30/PMA2 inserts, respectively, vs. 25.0 and 25.2 μg/ml for the RSI-15a/PAA and RSI-15a/PMA ones, respectively). Similar pulsed profiles were observed for RSI-10/PAA2 and RSI-15b/PAA2, which produced a C_{max} in tear fluid respectively of 74.5 and 73.3 μg/ml, 0.25 and 1.0 h after administration. The tear film OXT concentration pro-

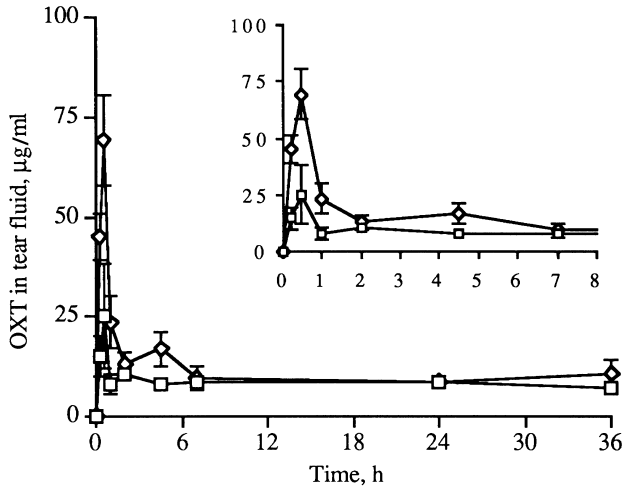

Fig. 5. OXT concentration profiles in tear fluid of rabbits after administration of twice-grafted RSI. (□) RSI-15a/PMA2; (◇) RSI-30/PMA2. For clarity, the release profiles during the first 8 h are reported in the inside graph. Vertical bars represent SE (n = 6).

Table 4

OXT release parameters in vivo

Insert type	C_{max} ± SE (μg/ml)	t_{max} (h)	$C_{plateau}$ (μg/ml)	$t_{plateau}$ (h)	$AUC_{0.25 \rightarrow 36h}$ ± SE (h·(μg/ml))
RSI-10/PAA2	74.5 ± 10.3	0.25	26.5	71.0	818.9 ± 134.1
RSI-15a/PAA2	33.0 ± 4.8	4.00	22.2	68.0	961.3 ± 161.0
RSI-15b/PAA2	73.3 ± 14.0	1.00	31.2	70.0	1273.4 ± 112.4
RSI-30/PAA2	113.8 ± 3.3	0.50	26.4	68.0	1111.8 ± 254.5
RSI-15a/PMA2	25.2 ± 13.1	0.50	8.3	35.0	300.8 ± 25.4
RSI-30/PMA2.	69.4 ± 11.3	0.50	13.7	35.0	393.7 ± 89.3

duced by PAA-grafted inserts in the 'plateau' period was higher with respect to that produced by PMA-grafted ones ($C_{plateau}$ 22.2–31.2 μg/ml for PAA-grafted inserts vs. 8.3–13.7 μg/ml for PMA-grafted ones). The release burst, more evident in the case of the more hydrophilic PAA-grafted inserts, might be put into relation with the initial hydration rate of the matrices, favoring a faster release of OXT through water-filled pores. The approximately constant OXT concentrations in tear fluid observed at longer times might depend on the establishment of an appropriate balance between drug release and physiological elimination mechanisms.

The minimum inhibitory concentration values (MIC 90%) of OXT observed in vitro against the micro-organisms responsible of common ocular infections range from 0.8 to 2.0 μg/ml, while MIC 90% values in the range 14-50 μg/ml have been indicated for *Pseudomonas aeruginosa* [15]. All of the presently described grafted inserts would ensure a prolonged OXT release in tear fluid with 'plateau' concentration values 10/30-fold higher with respect to the MIC 90% for common micro-organisms. Based on analogous assumptions in the literature [21,22] these concentrations should be sufficient to reach a therapeutic level.

The OXT bioavailability in tear film, as defined by the area under curve (AUC) values, was higher for the PAA2-grafted matrices with respect to PMA2-grafted ones. In particular, in the case of the PMA-grafted inserts the OXT bioavailability did not apparently depend on drug load (15% OXT, AUC = 300.8 h·(μg/ml); 30%, AUC = 393.7 h·(μg/ml), while for the PAA-grafted ones the bioavailability increased with increasing drug load (10% OXT, AUC = 818.9 h·(μg/ml); 30% OXT, AUC = 1111.8 h·(μg/ml)).

4. Conclusion

An ideal chemotherapy for ocular bacterial infections, such as trachoma (a major cause of blindness in the rural populations of developing countries) would require a sustained, constant-rate delivery of antibiotics to produce concentrations significantly above the MIC of ocular pathogens. Indeed, in contrast to bacteria, *chlamydia trachomatis* has a long life cycle of about 3–4 days. It has been reported that while doxycycline given orally approaches effective concentrations in tears, tetracycline and oxytetracycline given by the same route do not achieve inhibition level in these fluids [16]. An evaluation on rabbits of topically applied tetracycline in different vehicles showed that ocular levels of drug were highest with a petrolatum mineral oil-ointment and lowest with isotonic saline vehicles [17]. However, standard ophthalmic ointments are characterized by a pulsed delivery and typically require twice daily applications for several weeks [18] while ocular inserts might more efficiently produce the desired therapeutic results [19]. Gurtler et al. recently reported interesting results obtained with a 'Bioadhesive Ophthalmic Drug Insert' (BODI) containing gentamicin, which ensured an efficacious drug concentration in tears for 72 h [20,21].

The presently described mucoadhesive silicone inserts might likewise prove efficient platforms for delivery of antibiotics to the eye. As shown in this report, sustained release of OXT, zero-order release kinetics and prolonged retention in the conjunctival sac of rabbits could be obtained by suitably adjusting the matrix components and the thickness and type of IPN layer.

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