ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 50 (2000) 251–255

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

A poly(ortho ester) designed for combined ocular delivery of dexamethasone sodium phosphate and 5-fluorouracil: subconjunctival tolerance and in vitro release

M. Zignani[a], S. Einmahl[a], V. Baeyens[a], E. Varesio[b], J.L. Veuthey[b], J. Anderson[c], J. Heller[d], C. Tabatabay[a], R. Gurny[a,*]

[a]*Department of Pharmaceutics and Biopharmaceutics, School of Pharmacy, University of Geneva, Geneva, Switzerland*
[b]*Laboratory of Pharmaceutical Analytical Chemistry, University of Geneva, Geneva, Switzerland*
[c]*Institute of Pathology, Case Western Reserve University, Cleveland, OH, USA*
[d]*Advanced Polymer Systems, Redwood City, CA, USA*

Received 14 February 2000; accepted in revised form 17 April 2000

Abstract

A viscous hydrophobic poly(ortho ester) (POE) has been developed as a biocompatible, biodegradable sustained release system for selected cases of glaucoma filtering surgery. Dexamethasone and 5-fluorouracil (5-FU) are frequently administered together post-operatively, for their anti-fibroblastic and anti-inflammatory properties, respectively. A combined sustained release of both drugs could be advantageously used. Drug release kinetics were studied using specially designed thermostated cells. Subconjunctival tolerance was evaluated on New Zealand albino rabbits by clinical evaluation. Due to its basicity, the addition of dexamethasone sodium phosphate (DEX-P) stabilized the polymer and prolonged 5-FU in vitro release from 2 to 4 days. Both therapeutic agents were released concomitantly, according to a linear profile. The presence of 5-FU only slightly affected the overall subconjunctival tolerance of POE in rabbits, whereas the addition of DEX-P markedly improved POE tolerance by reducing the hyperemia of the conjunctiva to a minimal grade.

Keywords: Glaucoma filtering surgery; Poly(ortho ester); Bioerodible polymers; 5-Fluorouracil; Dexamethasone; In vitro release; Capillary electrophoresis; Biocompatibility

1. Introduction

Glaucoma filtering surgery consists in the production of a filtration fistula to facilitate outflow of aqueous humor from the anterior chamber to reduce intraocular pressure. However, a wound healing process can seal the surgical site and result in failure of the operation [1]. Fibroblast proliferation plays an important role in this wound healing and scarring process. Various pharmacological agents such as antimetabolites and anti-inflammatory steroids have been shown to effectively inhibit the growth of fibroblasts in vitro [2]. 5-Fluorouracil (5-FU) is a fluorinated pyrimidine analogue that inhibits fibroblast proliferation by competitive inhibition of thymidilate synthetase. It is widely used to increase the success of filtering procedures in patients with poor surgical prognoses [3]. Since the 5-FU half-life is less than 4 h in the anterior chamber following subconjunctival administration, post-operative therapy after glaucoma filtering surgery requires frequent administration, with subsequent patient discomfort and high risk of infection. Dexamethasone is a synthetic corticosteroid, which is widely used as post-operative topical treatment and also requires frequent instillation. Dexamethasone may also reduce the intraocular inflammation as well as the breakdown of the blood–ocular barrier in proliferative vitreoretinopathy (PVR) [4]. Similarly, 5-FU has also been successfully tested for the treatment of PVR [4], with no demonstrable retinal toxicity at doses up to 1.0 mg. However, frequent injections are required, because of a short intravitreal half-life. In both above-mentioned surgery procedures, a polymeric bioerodible carrier providing sustained release of both 5-FU and dexamethasone would

* Corresponding author. Department of Pharmaceutics and Biopharmaceutics, School of Pharmacy, University of Geneva, quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland. Tel.: +41-22-702-6146; fax: +41-22-7026567.

E-mail address: robert.gurny@pharm.unige.ch (R. Gurny).

0939-6411/00/$ - see front matter
PII: S0939-6411(00)00107-7

be appropriate in order to target different components of the wound healing process and to avoid repeated injections.

The potential use of a polymer is strongly dependent on its biocompatibility; a marked inflammatory reaction would counter the effect of the wound healing inhibitors. POE biocompatibility has been extensively investigated and markedly improved by purifying the polymer from residual monomers and oligomers, by producing the polymer aseptically [5] and by better controlling the local acidity generated during the polymer degradation [6]. In view of tissue response and biocompatibility, it is extremely important to consider the biological activity of the therapeutic agents incorporated into the carrier, especially if the bioactive agent has cytotoxic or anti-inflammatory characteristics. The inherent toxicity of therapeutic agents must be considered as having the potential of altering the inflammatory (phase I) and foreign body reaction (phase II) [7]. Previous studies have demonstrated that some drugs, such as naltrexone and cisplatin, adversely affect the histocompatibility, whereas hydrocortisone acetate decreases tissue response [8]. Moreover, the incorporation of therapeutic agents may modulate degradation rate of the polymer and also indirectly influence tissue response [7]. For these reasons, ISO standards 10993 regarding the biological evaluation of medical devices require various tests on the final product, i.e. the sterilized polymer carrier containing the therapeutic agents.

The present study evaluates the local tolerance of a POE carrier containing 5-FU, with and without dexamethasone. In vitro release studies and pH determination during polymer degradation are also presented.

2. Materials and methods

2.1. Polymer synthesis

As previously described by Merkli et al. [9], POE was synthesized by a transesterification reaction between 1,2,6-hexanetriol and trimethyl orthoacetate (Aldrich® Chemie, Steinheim, Germany). The reaction, catalyzed by *p*-toluene-sulfonic acid, was carried out by distilling the two precursors in cyclohexane (Fluka Chemie® AG, Buchs, Switzerland) under anhydrous conditions. POE was purified by a precipitation procedure to remove all impurities such as residual monomers and oligomers. To further eliminate residual solvents, the polymer was dried under vacuum (10^{-2} mbar) at room temperature. POE was produced aseptically using a 0.2 μm air filter (Millipore® GV) during solvent evaporation to exclude air contamination. This aseptic process has been successfully tested with different nutrient media (broad spectrum) [10].

2.2. Characterization of POE

The structure of POE was characterized by ^{1}H and ^{13}C-NMR spectroscopy [9]. The average molecular weight of the POE was determined by size exclusion chromatography (SEC) using a Waters® 150 CV instrument with three Ultrastyragel® (Waters®, Volketswil, Switzerland) columns 100, 500, 1000 Å pore size in series, and tetrahydrofuran (Romil® Chemicals, Leics, UK) as eluent [9].

2.3. In vitro pH monitoring during POE degradation

The pH was measured during POE degradation in order to monitor the decrease of pH induced by the release of acetic acid. In a sample flask, 1 g POE and 10 ml 0.9% sodium chloride (Fluka® Chemie AG, Buchs, Switzerland) solution or phosphate-buffered solution (PBS) (pH 7.4) were placed in a horizontal shaker (Haling®, Aigle, Switzerland) at 37°C operating at low speed (100 U/min). Measurements of pH were taken every day during the first week and then every week using a pH-meter Mettler DL25 (Nanikon-Uster®, Switzerland) and a combined pH-glass micro-electrode (6.0204.100Pc, Metrohm®, Herisau, Switzerland), until complete degradation [10].

2.4. Sample preparation

The samples were prepared under a laminar air-flow hood. The added drugs and excipients 5-FU and dexamethasone sodium phosphate (DEX-P) (Sigma® Chemical Co., St. Louis, MO) were previously γ-sterilized at 2.0 MRad, and were mixed with the polymer under aseptic conditions at room temperature [10].

2.5. In vitro release studies

Drug release studies were conducted in specially designed thermostated cells containing 200 mg of POE. DEX-P and/or 5-FU loaded polymer was placed into the cells, and PBS (pH 7.4) was circulated through the cells at the rate of 8 ml/h, and collected every 4 h using an automatic fraction collector 2111 Multirac (LKB®, Bromma, Sweden). The cell temperature was controlled with a D8 thermostatic controller (Haake®, Karlsruhe, Germany) [11]. The amount of 5-FU and DEX-P released was determined by a capillary zone electrophoresis assay [12]. Briefly, experiments were carried out on a HP^{3D} CE system (Hewlett-Packard®, Wilmington, DE) with a fused-silica capillary equipped with an extended path-length detection window. Detection was performed using a diode array detector scanning wavelengths from 190 to 600 nm. Electropherograms were monitored at 242 nm for DEX-P, 265 nm for 5-FU and 300 nm for indoprofen (internal standard).

Polymer weight loss was obtained gravimetrically: the polymer was removed when release was completed and the sample lyophilized with a Lyolab B II (Secfroid® SA, Aclens-Lausanne, Switzerland).

2.6. Subconjunctival injections

New Zealand albino rabbits were used and treated as approved by the Animal Care and Use Committee of the

University of Geneva. POE was injected subconjunctivally using a hydraulic syringe through a 20 G needle under local anesthesia, the injection volume amounting to 200 μl. The injections were performed nasally into the superior rectus muscle while the rabbits were in restraining boxes. Each product was tested on 3–6 rabbits. A slit-lamp examination was performed daily, and a 0 (absence) to 3 (highest) clinical evaluation scale scoring was used to grade, respectively, hyperemia and chemosis of the conjunctiva and episclera, as well as lachrymation induced by POE with the previously mentioned drugs [10]. Experimental eyes were compared with contralateral, non-injected eyes (score 0). Statistical comparison between each group was performed using a non-parametric Mann–Whitney *U*-test.

3. Results and discussion

3.1. In vitro pH determination

As previously reported [9], POE erodes gradually by surface hydrolysis, yielding first a mixture of esters of the initial triol, followed by a slower hydrolysis to 1,2,6-hexanetriol and acetic acid. Since the second hydrolysis is much slower, no autocatalysis is observed. When placed in saline solution, the in vitro pH determination, during POE degradation, shows a significant decrease from pH 6 to 2.5. However, in PBS solution, the pH is maintained between 7 and 5 [5,10]. In this case, the addition of the slightly acidic 5-FU does not influence the degradation rate nor the pH variation during POE degradation. POE presence in saline, with or without 5-FU, extended to 1 day and a similar pH decrease was observed. Due to the basicity of DEX-P, the final pH was stabilized around 3.2 instead of 2.5 and the polymer lifetime was prolonged to 2 days in saline solution and to 3 days in PBS.

3.2. In vitro release studies

The release of 5-FU from POE has been extensively studied by Merkli et al. [11]. The optimal drug concentration was determined to be around 1% (w/w), because of its water solubility and slightly acidic nature, which catalyzes polymer degradation. As shown in Fig. 1, the release of 5-FU as well as polymer erosion are completed within 72 h in vitro,with a 8 kDa POE.

As previously reported, the addition of a basic excipient

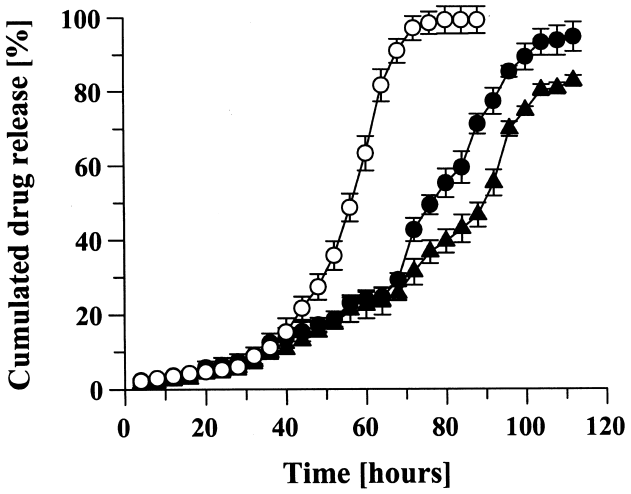

Fig. 1. Cumulative in vitro release of 5-FU (○) alone, or 5-FU (●) combined with DEX-P (▲) from POE (8 kDa) (phosphate buffer (pH 7.4); 37°C; $n = 6$, mean ± SD; drug loading 1% w/w).

stabilizes the ortho-ester bonds and reduces POE degradation rate [6,11]. The addition of basic DEX-P significantly prolongs 5-FU release from 3 to 4 days in vitro (Fig. 1). It is worth noting that even though 5-FU and DEX-P have different solubilities and pKas (Table 1), a concomitant release is observed. In the presence of both therapeutic agents, a good linearity of the release profiles is observed.

Interestingly, a similar linear release profile is observed after addition of 1% of highly insoluble magnesium hydroxide (MG) [6]. However, the addition of DEX-P shows a total polymer erosion when drug release is completed, whereas MG further prolongs the polymer lifetime another 10 days after release completion [6]. In the presence of MG, the release of 5-FU is thus controlled by diffusion, since 95% of the polymer is still present when the release is completed, whereas in the presence of highly soluble DEX-P, 5-FU release is mainly controlled by erosion. The difference of solubility and the basicity strength could explain this variation. The solubility of drugs incorporated into the polymer is also important, but this parameter seems to be of secondary importance [13]. In fact, the basicity of MG is higher than DEX-P. Also, the progressive acidification, triggered by the formation of acetic acid during polymer degradation could modify the respective solubilities. In fact, an acidic environment

Table 1
Chemical properties of therapeutic agents[a]

Therapeutic agents	Solubility (mg/ml)	pH of a 1% solution[b]	pKa_1	pKa_2	pKa_3	Molecular weight	Amount[c] (μmol)
5-FU	10	4.7	8	13	–	130.1	15.3
DEX-P	500	9.0	7.7	9.2	12.8	516.4	3.87

[a] 5-FU, 5-fluorouracil; DEX-P, dexamethasone disodium phosphate.
[b] In saline.
[c] In 200 μg of POE containing 1% w/w of drug.

reduces DEX-P solubility, whereas MG becomes soluble in presence of acetic acid.

3.3. Tolerance evaluation

Subconjunctival tolerance has been investigated in rabbit eyes after injection of 200 μl of an aseptically prepared POE containing 5-FU and/or DEX-P. The subconjunctival injection of POE alone triggered a hyperemia and a chemosis graded 1 during 3 days. The eye fully recovered by day 7 with no signs of chronic inflammation.

The addition of 5-FU did not markedly affect the overall tolerance of the POE carrier (Fig. 2). It triggered a similar hyperemia graded 1 during 3 days and a chemosis also graded 1 at day 1. However, the addition of 5-FU slightly enhanced conjunctival hyperemia between day 5 and day 7. A commercial 5-FU solution (Roche®) at 50 mg/ml has been similarly tested and induced an hyperemia and a chemosis graded 1.5 during the first 24 h. The pH of the solution required to dissolve 5-FU at 50 mg/ml is fairly high, around 9. Repeated injections of such a high pH solution may trigger severe local irritation and could explain the corneal defects frequently observed after 5-FU post-operative treatment. A sustained release rate of 5-FU could be advantageous in decreasing the local toxicity.

Comparatively, the addition of 1% DEX-P markedly improved the tolerance of POE. It reduced hyperemia of the conjunctiva to a minimal grade and completely suppressed any signs of conjunctival chemosis (Fig. 2). In fact, as an anti-inflammatory and immunosuppressive agent, it may inhibit edema, fibrin deposition, capillary dilatation and proliferation (angiogenesis), migration of leukocytes, fibroblast proliferation, deposition of collagen and scar formation [8]. DEX-P also prolonged the subconjunctival presence of the polymer for up to 8–10 days, whereas POE alone or containing 1% 5-FU was only present over 1–2 days. This can not be explained entirely by the stabilization of POE by the basic nature of DEX-P, because in vitro studies have shown only a slight increase in polymer lifetime from 1 to 2 days. The in vivo prolonged presence of polymer containing DEX-P could also result from a reduced inflammatory cell infiltration, notably a decreased accumulation of macrophages, due to the anti-inflammatory properties of DEX. Similarly, macrophages are the principal producers of a series of lysosomal enzymes, such as hydrolases or esterases [14], which could also trigger massive polymer cleavage. The prolonged in vivo presence of POE containing DEX-P [15] may indicate a possible longer in vivo drug release compared with in vitro predictions. It is interesting to note that the reduced accumulation of all types of leukocytes at the injection site, due to the corticosteroid activity, could also prevent polymer encapsulation. This point is particularly critical when the presence of the polymer is approximately 2 weeks long [8].

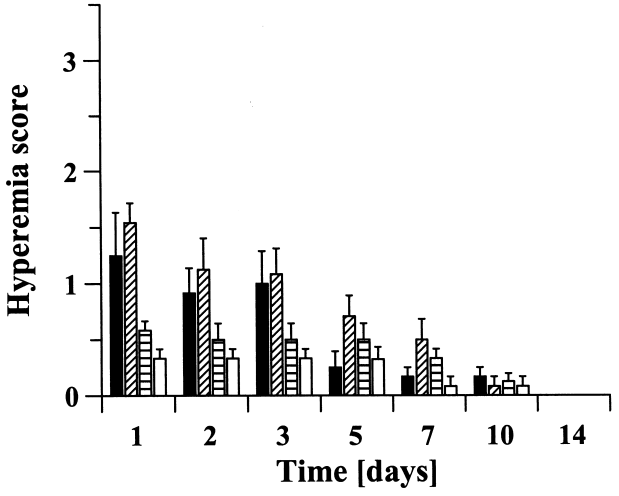

Fig. 2. Comparative hyperemia score of (■) POE (9.5 kDa) ($n = 6$), (▨) POE with 5-FU ($n = 6$), (▤) POE with DEX-P ($n = 3$), and (□) POE with 5-FU and DEX-P ($n = 3$). (Mean ± SD; drug loading 1% w/w).

The POE system containing both 5-FU and DEX-P has also been evaluated and was well tolerated. It only showed a slightly higher hyperemia, compared with the addition of DEX-P alone. Similarly, no chemosis was observed.

In no eyes of any group, lachrymation could be observed.

4. Conclusion

This study has clearly shown that the presence of 5-FU only slightly affects POE degradation rate and subconjunctival tolerance. The addition of DEX-P has several advantages: (i) its anti-inflammatory properties improve surgical prognoses as well as polymer tolerance; (ii) its basic nature decreases POE degradation rate and prolongs 5-FU release, maintaining a linear profile as well as a simultaneous polymer erosion and drug release.

Acknowledgements

This work was supported by Swiss National Science Foundation grant #32.46795.96.

References

[1] M.R. Chang, Q. Cheng, D.A. Lee, Basic science and clinical aspects of wound healing in glaucoma filtering surgery, J. Ocul. Pharmacol. 14 (1998) 75–95.

[2] M.S. Blumenkranz, A.J. Claflin, A.S. Hajek, Selection of therapeutic agents for intraocular proliferative disease. Cell culture evaluation, Arch. Ophthalmol. 102 (1984) 598–604.

[3] The Fluorouracil Filtering Surgery Study Group, Five-year follow-up of the fluorouracil filtering surgery study, Am. J. Ophthalmol. 121 (1996) 349–366.

[4] C.S. Yang, J.A. Khawly, D.P. Hainsworth, S.N. Chen, P. Ashton, H. Guo, G.J. Jaffe, An intravitreal sustained-release triamcinolone and 5-fluorouracil codrug in the treatment of experimental proliferative vitreoretinopathy, Arch. Ophthalmol. 116 (1998) 69–77.

[5] M. Zignani, A. Merkli, M.B. Sintzel, S.B. Bernatchez, W. Kloeti, J. Heller, C. Tabatabay, R. Gurny, New generation of poly(ortho esters):

synthesis, characterization, kinetics, sterilization and biocompatibility, J. Control. Release 48 (1997) 115–129.

[6] M. Zignani, T. Le Minh, S. Einmahl, C. Tabatabay, J. Heller, J.M. Anderson, R. Gurny, Improved biocompatibility of a viscous bioerodible poly(ortho ester) by controlling the environmental pH during degradation, Biomaterials 21 (17) 2000.

[7] J.M. Anderson, M.S. Shive, Biodegradation and biocompatibility of PLA and PLGA microspheres, Adv. Drug Del. Rev. 28 (1997) 5–24.

[8] K.L. Spilizewski, R.E. Marchant, C.R. Hamlin, J.M. Anderson, T.R. Tice, T.O. Dappert, W.E. Meyers, The effect of hydrocortisone acetate loaded poly(DL-lactide) films on the inflammatory response, J. Control. Release 2 (1985) 197–203.

[9] A. Merkli, J. Heller, C. Tabatabay, R. Gurny, Synthesis and characterization of a new biodegradable semi-solid poly(ortho ester) for drug delivery systems, J. Biomater. Sci. Polym. Edn. 4 (1993) 505–516.

[10] M. Zignani, S.B. Bernatchez, T. Le Minh, C. Tabatabay, J.M. Anderson, R. Gurny, Subconjunctival biocompatibility of a viscous bioerodible poly(ortho ester), J. Biomed. Mater. Res. 39 (1998) 277–285.

[11] A. Merkli, J. Heller, C. Tabatabay, R. Gurny, The use of acidic and basic excipients in the release of 5-fluorouracil and mitomycin c from a semi-solid bioerodible poly(ortho ester), J. Control. Release 33 (1995) 415–421.

[12] V. Baeyens, E. Varesio, J.L. Veuthey, R. Gurny, Determination of dexamethasone in tears by capillary electrophoresis, J. Chrom. B 692 (1996) 222–226.

[13] S. Einmahl, M. Zignani, E. Varesio, J. Heller, J.L. Veuthey, C. Tabatabay, R. Gurny, Concomitant and controlled release of dexamethasone and 5-fluorouracil from poly(ortho ester), Int. J. Pharm. 185 (1999) 189–198.

[14] G.N. Kumar, Drug metabolizing enzyme systems in the eye, in: I.K. Reddy (Ed.), Ocular Therapeutics and Drug Delivery. A Multi-disciplinary Approach, Technomic Publishing AG, Basel, 1996, pp. 149–167.

[15] S. Einmahl, F.F. Behar-Cohen, C. Tabatabay, M. Savoldelli, F. D'Hermies, D. Chauvaud, J. Heller, R. Gurny, A viscous bioerodible poly(ortho ester) as a new biomaterial for intraocular application, J. Biomed. Mater. Res. 50 (2000) 566–573.