Improved biocompatibility of a viscous bioerodable poly(orthoester) by controlling the environmental pH

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IMPROVED BIOCOMPATIBILITY OF A VISCOUS BIOERODABLE POLY(ORTHO ESTER) BY CONTROLLING THE ENVIRONMENTAL pH

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INTRODUCTION

A viscous biodegradable poly(ortho ester) (POE) carrier has been developed for drug release in glaucoma filtering surgery. Antifibroblastic agents, such as 5-fluorouracil (5-FU) are widely used to increase the success of filtering procedures in patients with poor surgical prognoses [1]. Since a marked inflammatory reaction would counter the effect of the wound healing inhibitor (5-FU), the potential use of the polymer is strongly dependent on its biocompatibility.

POE biocompatibility has been firstly markedly improved by purifying the polymer to remove residual monomers and oligomers [2,3]. Since it has been shown that gamma-sterilization partially degrades POE, the inflammatory reaction has been further reduced by producing the polymer aseptically [3,4]. The local toxicity of the initial monomers and different breakdown moieties have also been extensively investigated. Since the two initial monomers and the intermediate degradation products induced only a moderate inflammatory reaction, the acute inflammation has been attributed to the formation of an acidic by-product [3]. For this reason, a better control of the local acidity generated by the polymer degradation has been investigated. This study presents several approaches, which have been evaluated in order to reduce the inflammatory reaction by buffering or neutralizing the acidic by-product and by decreasing the degradation rate of the polymer and thus the release rate of the acidic moiety.

MATERIALS AND METHODS

Polymer synthesis

POE was synthesised by a transesterification reaction between trimethyl orthoacetate and 1,2,6-hexanetriol (Aldrich® Chemie, Steinheim, D), followed by a self-condensation of the reaction product [4,5]. POE was purified by precipitation and produced under aseptic conditions [4].

Polymer degradation

The ortho ester bonds easily hydrolyze with the formation of isomeric esters of the initial hexanetriol, followed by a slower hydrolysis to the original triol and acetic acid [4].

In vitro pH determination

The pH was measured during POE degradation in order to assess the decrease of pH induced by the formation of acetic acid. One g (w/w) POE was placed in 10 ml of 0.9% saline solution, in a shaker at 37°C under light shaking (100U/min) [4].

In vitro relase studies

Drug release studies were conducted in specially designed thermostated cells (37°C), with circulating phosphate buffer pH 7.4, at the rate of 10 ml/hr, and collected every hour using an automatic fraction collector 2111 Multirac (LKB®, Bromma, S). Each cell contains 200 mg of POE, with 1% (w/w) drug loading, prepared by simple mixing at room temperature. The amount of 5-FU (Sigma® Chemie AG, Buchs, CH) released was measured by UV at 266 nm with a diode array 8452A spectrophotometer (Hewlett-Packard®, Meyrin, CH). Polymer weight loss was measured gravimetrically. The polymer was removed and dried by lyophilization with a Lyolab B II (Secfroid SA, Aclens-Lausanne, CH), when the release was completed [4,5].

Subconjunctival injections

POE was injected subconjunctivally with a hydraulic syringe, through a 20G needle to New Zealand Albino rabbits, under local anaesthesia, according to the Animal Care and Use Committee of the University of Geneva. A clinical examinations was performed daily at the slit lamp. A clinical evaluation scale [0 (=absence) to 3+ (=highest)] was used to grade respectively the hyperemia of the conjunctiva and episclera, the chemosis and the lachrymation induced by POE with addition or not of different excipients. The results are presented according to the modified Draize's test based on the addition of the respective hyperemia, chemosis and lachrymation scores. Animals were sacrified for histological studies. Hematoxylin-eosin and Giemsa stainings were performed [3].

RESULTS AND DISCUSSION

The in vitro monitoring of the acetic acid formation showed a rapid decrease of the environmental pH during POE degradation (Fig. 1). For this reason, the acid formed has been first neutralized by adding $Mg(OH)_2$ which had previously been used to prolong drug release. $Mg(OH)_2$ initially rised the pH up to 9 (Fig. 1), and markedly prolonged the

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polymer life. We also studied another approach to overcome problems due to low pH environment, by buffering the formed acetic acid with its alkali salt: sodium acetate ($Na(CH_3COO)$) . The buffered POE showed an initial pH of 6, which subsequently stabilized around 5, and slightly affected polymer degradation rate.

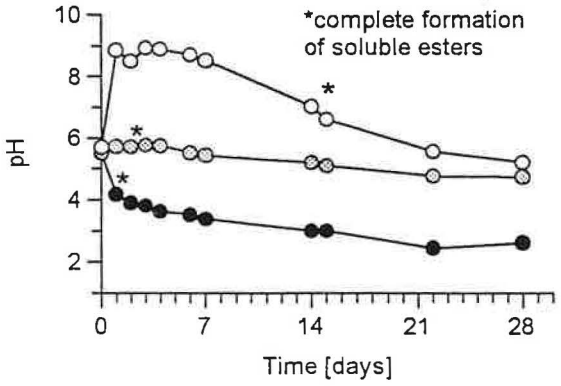

Fig. 1 In vitro pH profiles during degradation of (●) aseptically prepared POE (8kDa), (◎) +1% (w/w) $Na(CH_3COO)$; (○) +1% (w/w) $Mg(OH)_2$. (n=3, +/-sd)

The influence on drug release of the above-mentioned additives has been studied in vitro. As previously reported [5], the incorporation of $Mg(OH)_2$ markedly prolonged drug release by stabilizing the acid-labile bonds with its basic and hydrophobic nature. However, a greater divergence between polymer erosion and drug release has been noted. The addition of sodium acetate has shown very little effect on the rate of drug release (Fig. 2). This can be attributed to an equilibrium between the basic nature of sodium acetate and its hydrophilicity. Thus, its hydrophilicity leads to an increased water uptake of the polymer, while its basicity stabilizes the poly(ortho ester) acid-labile bonds.

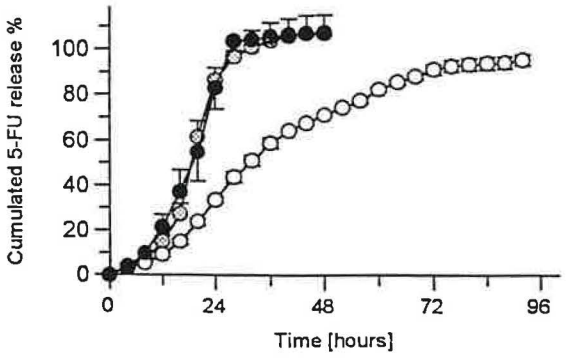

Fig.2 Cumulative in vitro release of 5-FU (1%w/w) from (●) aseptically prepared POE (8kDa),(◎)+1%(w/w) $Na(CH_3COO)$, (○) +1% (w/w) $Mg(OH)_2$ (n=6, +/-sd)

It is important to note that in glaucoma filtering surgery, polymer erosion over a maximum period of 10 to 15 days must be achieved in order to avoid polymer encapsulation [4].

According to the modified Draize's test, subconjunctival injection of POE only triggered a mild to a minimal irritation (Fig.3). The addition of ($NaCH_3COO$) mainly delayed the reaction, whereas the presence of $Mg(OH)_2$ slightly reduced the reaction and markedly prolonged the polymer life in situ.

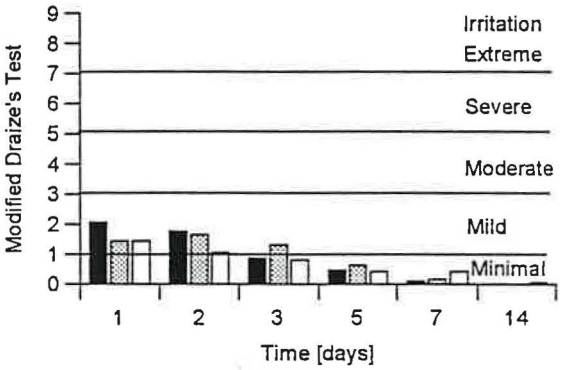

Fig 3 Comparative clinical evaluation of cumulated score according to Modified Draize's test induced by subconjunctival injection of (■) aseptically prepared POE (8 kDa) (n=9),(▨)+1% (w/w) $Na(CH_3COO)$ (n=12), (□) +1%(w/w) $Mg(OH)_2$ (n=6).

Histological sections of the cornea, the limbus and the sclera showed that the acute inflammatory reaction was resolved within 7 to 10 days, with no evidence of chronic inflammation, in the case of POE alone and with $Na(CH_3COO)$. In the case of $Mg(OH)_2$, rare multinucleated giant cells were observed after 14 days.

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