A New Poly(Ortho Ester)-Based Drug Delivery System as an Adjunct Treatment in Filtering Surgery

Suzanne Einmahl, Francine Bebar-Cohen, François D’Hermies, Serge Rudaz, Cyrus Tabatabay, Gilles Renard, and Robert Gurny


table

Purpose. Pharmacologic modulation of wound healing after glaucoma filtering surgery remains a major clinical challenge in ophthalmology. Poly(ortho ester) (POE) is a biodegradable and biocompatible viscous polymer potentially useful as a sustained drug delivery system that allows the frequency of intraocular injections to be reduced. The purpose of this study was to determine the efficacy of POE containing a precise amount of 5-fluorouracil (5-FU) in an experimental model of filtering surgery in the rabbit.

Methods. Trabeculectomy was performed in pigmented rabbit eyes. An ointmentlike formulation of POE containing 1% wt/wt 5-FU was injected subconjunctivally at the site of surgery, during the procedure. Intraocular pressure (IOP), bleb persistence, and ocular inflammatory reaction were monitored until postoperative day 30. Quantitative analysis of 5-FU was performed in the anterior chamber. Histologic analysis was used to assess the appearance of the filtering fistula and the polymer’s biocompatibility.

Results. The decrease in IOP from baseline and the persistence of the filtering bleb were significantly more marked in the 5-FU–treated eyes during postoperative days 9 through 28. Corneal toxicity triggered by 5-FU was significantly lower in the group that received 5-FU in POE compared with a 5-FU tamponade. Histopathologic evaluation showed that POE was well tolerated, and no fibrosis occurred in eyes treated with POE containing 5-FU.

Conclusions. In this rabbit model of trabeculectomy, the formulation based on POE and containing a precise amount of 5-FU reduced IOP and prolonged bleb persistence in a way similar to the conventional method of a 5-FU tamponade, while significantly reducing 5-FU toxicity. (Invest Ophthalmol Vis Sci. 2001;42:695–700)

Glaucoma filtering surgery involves producing a filtration fistula to allow controlled escape of aqueous humor from the surgical trabeculectomy into the subconjunctival space. The body’s natural tendency to heal, however, can seal the surgical trabeculectomy into the subconjunctival space. The decrease in IOP from baseline and the persistence of the filtering bleb were significantly more marked in the 5-FU–treated eyes during postoperative days 9 through 28. Corneal toxicity triggered by 5-FU was significantly lower in the group that received 5-FU in POE compared with a 5-FU tamponade. Histopathologic evaluation showed that POE was well tolerated, and no fibrosis occurred in eyes treated with POE containing 5-FU.

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Corresponding author: Robert Gurny, Department of Pharmaceutical Technology and Biopharmaceutics, School of Pharmacy, University of Geneva, Switzerland.

The antimetabolite 5-FU is a fluorinated pyrimidine analogue that competitively inhibits thymidylate synthetase, resulting in thymidine deficiency and decreased DNA synthesis. Currently, 5-FU is administered by frequent subconjunctival injections away from the surgical site. Such frequent 5-FU injections are inconvenient for the surgeon and uncomfortable for the patient. Furthermore, therapeutic drug levels are only transiently achieved, necessitating frequent injections and administration of large amounts of drug. Toxic effects on the surrounding ocular tissues are a predictable consequence of this dosage regimen. Toxicity on the conjunctival and corneal epithelium, wound dehiscence, and wound leaks have been reported after repeated subconjunctival administration of 5-FU.

Topical intraoperative administration of 5-FU with a sponge soaked in a concentrated drug solution has also been used and is associated with adverse side effects, some of which are vision threatening. Another disadvantage of this mode of administration is the high drug concentration to which the tissues are exposed during the tamponade as well as the imprecision of the amount of 5-FU delivered to the tissues.

These complications may be reduced by using subconjunctivally implanted drug delivery systems that would provide a localized and sustained release of antiproliferative drugs over an extended period (approximately 2 weeks) and then would disappear, leaving a patent fistula after glaucoma filtering surgery. This 2-week period is critical with respect to inflammatory and fibrotic reactions. Drug delivery systems investigated to date include collagen implants, bioerodible polymers, nonbioerodible polymers, liposomes, and microparticles.

Poly(ortho esters) (POE) are a new family of hydrophobic, biocompatible, and biodegradable polymers possessing particularly interesting characteristics. Biodegradability represents a significant advantage over other drug delivery systems, because there is no need to remove the device surgically once all the drug has been released. Kinetics of drug release from POE, as well as polymer degradation rate, are almost constant, without any burst effect, and can be controlled by factors such as polymer molecular weight and the physicochemical properties of the incorporated substances. The viscous, ointmentlike consistency of the polymeric material allows the incorporation of drugs into the carrier by simple mixing at room temperature, without the use of solvents. POE can be injected using a conventional syringe with an appropriate needle, which is a significant advantage when compared with solid devices that must be placed either with a trocar or through a more complex surgical procedure. Moreover, the viscous properties of POE make its presence within the eye smooth and nontraumatic, because it spreads in the space.

The purpose of this study was to evaluate a sustained drug delivery system based on POE and 5-FU as an adjunct treatment to filtering surgery in rabbits.
Materials and Methods

Polymer Synthesis

As described earlier,23 POE is synthesized by a transesterification reaction between 1,2,6-hexanetriol and trimethyl orthoate (Aldrich Chemie, Steinheim, Germany) under anhydrous and asptic conditions. POE is further purified by a precipitation procedure in methanol to remove impurities such as residual monomers and oligomers. The polymer was characterized by infrared spectroscopy, nuclear magnetic resonance and size exclusion chromatography.22 The average molecular weight of the polymer used in this study is 9.3 kDa, with a polydispersity of 1.4. POE is a viscous material, with Newtonian behavior. It is transparent, with a refractive index of 1.47 and is gel-like in appearance.

Preparation of the Formulations

Formulations were prepared under a laminar air-flow hood. The added drug, 5-FU (Sigma, Buchs, Switzerland), had been γ-sterilized at 2.0 MRad and homogeneously dispersed in the aseptically prepared semi-solid polymer under asptic conditions at a concentration of 1% wt/wt.24 The viscous mixture was conditioned into a 1.0-ml syringe, each sample being 200 µl (240 mg).

Animals

Pigmented Fauve de Bourgogne female rabbits weighing from 2 to 3 kg, 10 to 12 weeks of age, were used (Jean-Pierre Ravaud; Institut de la Recherche Agronomique, Nouzilly, France), and experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Six eyes were used in each experimental group.

Filtering Surgery

General anesthesia was induced with intramuscular injection of 50 mg/kg ketamine and 15 mg/kg xylazine. With rabbits under local anesthesia with oxybuprocaine 0.4% (Novesine; Ciba Vision, Basel, Switzerland), a lid speculum was inserted to expose the globe. A 45° blade that entered the anterior chamber. A block of tissue containing inner sclera, trabeculum and peripheral cornea, measuring approximately 3 × 1 mm, was excised at the limbus. A lid speculum was inserted to expose the globe. A 45° blade that entered the anterior chamber. A block of tissue containing inner sclera, trabeculum and peripheral cornea, measuring approximately 3 × 1 mm, was excised at the limbus. A lid speculum was inserted to expose the globe. A 45° blade that entered the anterior chamber. A block of tissue containing inner sclera, trabeculum and peripheral cornea, measuring approximately 3 × 1 mm, was excised at the limbus. A peripheral iridectomy was then performed. The scleral flap was approximated with two 10-0 nylon sutures. The conjunctiva was repositioned and the wound closed with 8-0 Vicryl suture (Ethicon, Piscataway, NJ) in a continuous fashion. Just before the last step, a 0.9-mm needle (20-gauge) was inserted in the subconjunctival space, and 200 µl (240 mg).

Histologic Analysis

Rabbits were killed at different time points ranging from 5 to 30 days after surgery by intracardiac injection of a lethal dose of pentobarbital, and their eyes were enucleated and fixed to be studied histologically by conventional optical microscopy. Anteroposterior sections were stained with hematoxylin-eosin to examine the conjunctiva, the irido-corneal angle, and the appearance of the site of the bleb. Every sample was treated simultaneously to reduce variations among fixation procedures.

Results

Clinical Findings

Clinical Appearance of the Eyes. Eyes were observed clinically for 30 days with special attention to conjunctival hyperemia and corneal edema. Results are summarized in Figure 1. Conjunctival hyperemia was scored according to a modified Draize test19: grade 0, normal vessels; grade 1, definitely injected vessels; grade 2, diffuse crimson red, individual vessels not easily discernible; and grade 3, diffuse beefy red. Corneal edema was scored as follows: grade 0, normal cornea; grade 1, slight corneal edema present at the surgical site; grade 2, diffuse corneal edema extending to half the surface of the cornea; and grade 3, opaque cornea with neovascularization.

Trabeculectomy triggered slight hyperemia of the conjunctiva that resolved after approximately 1 week. When 5-FU was applied as a tamponade, no significant increase of the hyperemia occurred. Hyperemia triggered by the presence of POE, with or without 5-FU (groups III and IV), had a score approximately 0.25 higher than in group I, with a significant difference at day 5. Eventually, hyperemia disappeared as the polymer degraded, by 2 weeks after surgery.19

At the site of surgery, trabeculectomy also triggered reversible edema of the cornea, which reached its apex at day 5. The
presence of POE triggered edema of the same intensity, which was also reversible. In contrast, 5-FU applied during surgery as a tamponade triggered more severe edema, extending to half the cornea (Fig. 2, II). The frequency and the severity of corneal edema in the 5-FU tamponade group was significantly higher than in any other group. Superficial punctuate keratitis occurred in two eyes. The group that received POE + 5-FU also showed some edema, but it remained localized at the surgical site and was not significantly different from that in the control groups (Fig. 2, IV).

POE could be observed at the subconjunctival site for 12 days, but the incorporation of the acidic 5-FU into the POE active formulation reduced its presence to 10 days because of accelerated, acid-catalyzed, degradation. After that time, POE seemed to disappear from the eye after bioresorption. It is possible that some POE may still have been present under the conjunctiva, but not visibly so, with the viscous POE spreading in the subconjunctival space. Moreover, it was sometimes difficult to distinguish a POE bubble from the filtering bleb.

Bleb Appearance and Survival Curves. In group I (control eyes that underwent trabeculectomy alone), all blebs were elevated and typically had sharply demarcated edges a few days after surgery. Fifty percent of blebs had collapsed by 9 days and 100% by 19 days. In group II (eyes that received an intraoperative 5-FU tamponade), the blebs were significantly more persistent, lasting from day 12 until the end of the experiment (day 29). Group III, which received POE alone, showed delayed persistence of the bleb compared with group I (Fig. 2, I and III), but eventually all blebs failed. All blebs in group IV (POE containing 5-FU) survived until postoperative day 19, and 83% survived after 1 month. There were no significant differences between group IV and group II. Bleb survival curves for the four groups are displayed in Figure 3.

Intraocular Pressure. In group I, the mean IOP returned to preoperative levels by day 9 (Fig. 4). In eyes that received polymer alone (group III), IOP also returned to baseline values, but with some delay. There was no statistical difference between these two control groups at any time.

Eyes treated with 5-FU either as a tamponade (group II) or incorporated in POE (group IV), had significantly lower IOP than control groups from day 9 until the end of the experi-

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**FIGURE 1.** Evaluation of conjunctival hyperemia and corneal edema. Group I (■), group II (□), group III (□), and group IV (□). n = 6, mean ± SD. *Significant difference from group I (P < 0.05).

**FIGURE 2.** Eye status in each group at postoperative day 7.
Quantitative Determination of 5-FU

The quantitative determination of 5-FU concentration in the aqueous humor is particularly important to ensure that the levels reached are not toxic to the corneal endothelium. Corneal toxicity includes epithelial defects, corneal opacification, and vascularization.

In all eyes that received POE + 5-FU, quantitative determination of 5-FU in the aqueous humor showed detectable amounts of 5-FU (below 0.5 μg/ml) for 2 weeks, which shows that POE was still present in the subconjunctival space for that period and released 5-FU in a continuous fashion. In particular, no burst release of 5-FU was observed in the early postoperative period, according to observations for in vitro 5-FU release. Thus, at each time point, a minimal amount of 5-FU was present in the anterior chamber, corresponding at least to 3 orders of magnitude below the threshold concentration for 5-FU toxicity to the corneal endothelium (1-10 mg/ml) reported by Mannis et al.

5-Fluorouracil was not quantified in the anterior chamber of eyes that received an intraoperative tamponade for several reasons: 5-FU is applied and rinsed off before opening the anterior chamber, and therefore no 5-FU should penetrate into the eye; 5-FU soaks the surrounding tissues and does not diffuse into the anterior chamber; moreover the flux direction is opposite to the direction of the possible 5-FU intracameral penetration; and last, in all the published literature concerning 5-FU intraoperative tamponade, none of the authors ever measured the 5-FU concentrations in the anterior chamber.

Histology

In control animals killed at 5 days after surgery, a bleb was present, and the trabeculectomy was patent. Some inflammatory cell (polymorphonuclear cells) infiltration was observed around the margins of the scleral flap. The edges of the iris around the iridectomy had partially prolapsed into the fistula in some specimens, although they did not completely block it. No evidence of any conjunctival filtration could be seen.

Bleb failure occurred at 10 days in eyes that underwent trabeculectomy alone (group I). Eyes showed evidence of inflammatory cell infiltration and fibrovascular tissue at the surgical site. The trabeculectomy and bleb closed due to bulk filling by granulation tissue and fibroblasts.

Until the end of the experiment, eyes that received 5-FU, either as a tamponade or incorporated in POE, showed no signs of fibrosis of the filtration fistula. The scleral flap margins were absent from any fibrotic subconjunctival fibroblasts (Fig. 6). The conjunctival filtration was efficient, visible as a small cavity surrounded by fibrin and cells. Some slight modifications of the iris were observed, notably pigment redistribution. These modifications of the uvea were more marked in the 5-FU tamponade group in which significant pigment alterations and pigment migration into the external tunica were observed.

Eyes were also observed to detect any inflammatory reaction triggered by POE. A transient acute inflammatory reaction was observed in the subconjunctival space, with granulated neutrophils around POE by day 5, as previously described. This inflammatory reaction was resolved within 2 weeks, with neither recurrence nor encapsulation of the biomaterial. In some eyes, giant multinucleated cells were found around cavities that were empty or filled with some grayish material, probably POE. No histologic evidence of chronic inflammation.
may have altered the inflammatory reaction. The administration of POE containing 5-FU promoted the success of glaucoma filtering surgery in rabbit eyes. Eyes that received POE and 5-FU after trabeculectomy had lower IOP for an extended period, with the trabeculectomy closing and the bleb collapsing within approximately 2 weeks, in contrast to PLGA-based systems, which show comparable results in IOP and bleb persistence. The incorporation of 5-FU in POE also reduced the toxicity of the drug, because lower amounts had to be used. Intraoperative 5-FU had a marked effect on IOP that lasted longer than is reported in the literature. The overall pattern of wound healing is similar to scars found in humans, although more rapid. It has also been reported that a significant reduction in IOP may occur, even in the absence of a patent trabeculectomy, and bleb function is thus a more reliable index to assess the fistula’s efficacy. Also, there are numerous anatomic differences between rabbit and human eyes. Notably, eyeball motion is more frequent in man, eyelid pressure is higher, and the iris is less prone to incarcerate the channel in rabbits than in man, so the potential for the mixture to ooze into the anterior chamber is higher in patients. However, we have seen that the concentrations obtained in the rabbit ante-
rior chamber after the administration of the POE 5-FU formulation were $10^3$ times lower than reported toxic concentrations. As a consequence, it is improbable that 5-FU concentrations in the human anterior chamber would increase 3 orders of magnitude; however, this should be investigated.

This newly developed drug delivery system based on biodegradable POE has potential for clinical applications in glaucoma filtering surgery in patients who are at high risk of failed surgery. Scarring at the filtration site can be inhibited by a localized and sustained release of a very small yet efficient amount of the antifibrobiastic agent 5-FU, therefore avoiding the need for frequent subconjunctival injections and decreasing toxic ocular side effects caused by intraoperative topical administration of higher amounts of drug. POE combines the advantage of a hydrophobic polymer, allowing a slow release of the hydrophilic drug 5-FU for 2 weeks, with a relatively fast biodegradability. Its proven biocompatibility makes it a biomaterial of choice to deliver drugs in a sustained, controllable way. A study over 6 months is currently under way to assess a long-term follow-up.

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References