The Use of Bioerodible Polymers and 5-Fluorouracil in Glaucoma Filtration Surgery

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A study was performed to examine the effect of a localized and sustained delivery of 5-fluorouracil (5-FU) on the success of glaucoma filtration surgery in 18 rabbits in a prospective, randomized, double-masked and placebo-controlled fashion. A bioerodible polyanhydride composed of bis (p-carboxyphenoxy) propane and sebacic acid was used as the drug carrier. The polymer and 5-FU (20% by weight) were compressed into 3 mm diameter discs, 1 mm thick. The polymer with the 5-FU was randomized to one eye and the fellow eye received the blank polymer. The results showed that intraocular pressures (IOP) were lower in the experimental eyes during the 5th through 17th postoperative days, but eventually both experimental and control eyes returned to preoperative levels. Filtration blebs lasted longer in experimental eyes when compared to control eyes. Implant disappearance occurred after IOP elevations and bleb failure. Eventually, the filtration surgery failed in both the experimental and control rabbit eyes. Invest Ophthalmol Vis Sci 29:1692-1697, 1988

The antimetabolite 5-fluorouracil (5-FU), a fluorinated pyrimidine which competitively inhibits thymidylate synthetase, resulting in an inhibition of deoxyribonucleic acid (DNA) synthesis, has been found to improve the success of glaucoma filtration surgery in the eyes of nonhuman primates1 and in the eyes of glaucoma patients with poor surgical prognoses.2,3 The presumed mechanism for the increase in success rate is the inhibition of fibroblast proliferation and subsequent scarring at the site of surgery by 5-FU. Subconjunctival administration of 5-FU postoperatively may improve the success rate of glaucoma filtration surgery, but also has the disadvantages of frequent administration, patient discomfort, and ocular surface problems.4 These disadvantages may be eliminated by different drug delivery systems which provide a controlled and localized release of drug over an extended period of time.5-7 Drug delivery systems using a collagen implant8 or bioerodible polymers9 have been investigated. This paper presents the results of a series of experiments using 5-FU impregnated bioerodible polymer discs in a rabbit model of glaucoma filtration surgery.

Materials and Methods

Chemicals were obtained as follows: 5-fluorouracil from Sigma Chemical Company (St. Louis, MO); ketamine from Parke-Davis (Morris Plains, NJ); xylazine from Haver-Lockhart (Shawnee, KA); pento-barbital from Abbott Laboratories (North Chicago, IL); paraformaldehyde, glutaraldehyde and sodium cacodylate from Tousimis (Rockville, MD). All other chemicals were reagent grade. All animal subjects were treated in accordance to the ARVO Resolution on the Use of Animals in Research.

The copolyanhydride of bis (p-carboxyphenoxy) propane (PCPP) and sebacic acid (SA), in a ratio of 50:50 by weight, was used as the carrier matrix. The polymers were synthesized by adapting the method described by Conix.10 The implants were fabricated by compressing the polymer (100 Kpsi and room temperature) combined with solid 5-FU (20% by weight) into discs of the dimension 3 mm in diameter and 1 mm thick. Each implant therefore contained 1.5 ± 0.1 mg of 5-FU. The control implants were made in an identical manner in the absence of 5-FU. All implants were stored desiccated in a bottle.

A prospective, randomized, double-masked and placebo-controlled study was performed by comparing a bioerodible polymer impregnated with 20% 5-FU to the same bioerodible polymer without any drug. The intraocular pressures of 18 normal Dutch rabbits, each weighing between 1.5 and 2.5 kg, were...
measured by pneumotonometry (Alcon Applanation Pneumatonograph, Digilab Inc., Cambridge, MA). All of the rabbits underwent preoperative eye examinations with a Kowa hand-held slit-lamp biomicroscope. Prior to each intraocular pressure measurement, one drop of 0.5% proparacaine hydrochloride was topically applied to each eye.

General anesthesia was given using ketamine 50 mg/kg I.M. and xylazine 15 mg/kg I.M. A posterior lip sclerectomy was performed on both eyes of each animal by the same surgeon. A lid speculum was inserted and the superotemporal conjunctiva was incised near the fornix with Wescott scissors. The conjunctiva was carefully dissected anteriorly to the limbus. Excessive Tenon's tissue overlying the sclera was excised. A limbal groove was made with a 57 Beaver blade and extended anteriorly into the corneal stroma. Before the anterior chamber was entered, a paracentesis was made through peripheral clear cornea away from the filtering site. Then the anterior chamber was entered through the filtering site and a 1 x 3 mm block of scleral tissue and trabecular meshwork was excised with Vannas scissors. The edges of the sclerectomy were cauterized to control hemostasis. Then a peripheral iridectomy was performed. The disc-shaped polymer was placed over the sclera adjacent to the sclerostomy site in the subconjunctival space (Fig. 1). Both eyes of each rabbit had the polymer inserted, the experimental eye had the polymer containing 5-FU and the control eye had the polymer without any 5-FU. The polymer with 20% 5-FU was randomly assigned to one eye and the fellow eye received the control polymer without the drug. The conjunctival incision was closed over the polymer with a running 10-0 nylon suture (Fig. 2). The anterior chamber was reformed with sterile saline solution through the paracentesis site and the bleb was checked for leaks. At the conclusion of the surgical procedure, topical erythromycin ointment was applied to both eyes of each animal in order to minimize the risk of postoperative infection.

Postoperative follow-up included ocular examinations with a Kowa hand-held slit-lamp biomicroscope and intraocular pressure measurements by pneumotonometry the first day after surgery and every other day thereafter for 3 weeks. Observations were recorded on individualized charts for each animal, with special attention given to the appearance of the bleb, conjunctiva, implant, cornea and anterior chamber.

The animals were sacrificed with an overdose of sodium pentobarbital 75 mg/kg I.V. Two animals were sacrificed 2 weeks after surgery and 16 animals were sacrificed 5 weeks after surgery. The two animals which were sacrificed 2 weeks after surgery were enucleated and the eyes were processed for light microscopy.

A similar but separate series of ten Dutch rabbits were studied to compare the effects of glaucoma filtration surgery with the disc polymer without drugs to glaucoma filtration surgery without any polymer. This series of ten animals was followed in an identical manner.

The eyes of two study animals (four eyes) were enucleated after sacrifice and fixed in 10% neutral buffered formalin for 48 hr prior to sectioning. The eyes were sectioned near the sclerostomy site. The cut specimens were dehydrated, infiltrated, and embedded in paraffin. Eight micron step sections of the bleb (wound) were cut with a rotary microtome. Sections were prepared with hematoxylin and eosin and Mas-
Statistically significant lower pressures occurred in experimental eyes between postoperative days 5 through 17.

Figure 3. Graph of postoperative intraocular pressures over time.

The in vitro drug release kinetics were determined by placing the drug-loaded discs in vials containing 10 ml of 0.1 M phosphate buffer, pH 7.4, at 37°C. The periodically changed buffer solutions were then subjected to HPLC analysis as described previously. Statistical analysis was done to compare experimental eyes with the fellow control eyes, using the paired t-test, paired Wilcoxon test, and McNemar's test, as appropriate for different variables.

Fig. 5. Photomicrograph of a histologic section from the sclerostomy site of a rabbit eye with a polymer containing 5-FU. Cornea (C), ciliary body (CB), and open sclerostomy (arrow) are shown (Masson’s trichrome, original magnification ×48).
following variables were analyzed: intraocular pressure, time to bleb failure, time to implant disappearance, conjunctival injection, corneal haze and pigmentation, and hyphema. A probability of less than 0.05 was considered to be statistically significant.

**Results**

Of the 18 rabbits in this study, postoperative hyphemas occurred in three of the 5-FU eyes and two of the control eyes. The polymer was extruded in only one eye, which had contained the control polymer. There were no intraoperative complications of conjunctival button holes or vitreous loss. There were no postoperative complications of wound leaks, corneal haze or pigmentation or endophthalmitis.

The postoperative pressures are shown in Figure 3. From day 5 to day 17 after surgery the intraocular pressures in the experimental 5-FU eyes were significantly lower than in the control eyes. However, eventually the intraocular pressures returned to their preoperative levels in both eyes. The blebs in the 5-FU eyes lasted significantly longer than in the control eyes. There was no significant difference in the rate of implant disappearance between experimental and control eyes, nor was there a temporal relationship between implant disappearance and the return of the intraocular pressure to preoperative levels. The polymer lasted during the entire five week follow-up period. Eventually, filtration surgery failed in both experimental and control eyes.

There was no difference in the postoperative intraocular pressure course in the eyes which received a control polymer disc and fellow eyes which received no disc (Fig. 4). The intraoperative and postoperative complication rates between the experimental and control eyes were identical.

Four representative eyes of the 18 rabbits which had the polymer with 5-FU in one eye and the blank polymer in the fellow eye were harvested 14 days after surgery and studied by light microscopy. Sclerotomy sites were identified. The 5-FU treated eyes had open sclerostomies (Fig. 5), while the control eyes had sclerostomy sites occluded by granulation tissue (Fig. 6). Due to difficulty in separating the implant completely from the fibrotic tissue, we could only esti-
mate the extent of matrix degradation. The weight loss of the carrier was observed to be 12% ± 8%. The determination of the 5-FU remaining in the matrix was plagued by the inability of the implant to completely dissolve in a good solvent such as chloroform; the implants were then extracted with acetonitrile, which is a good solvent for 5-FU, but a nonsolvent for the polymer. The HPLC analysis showed that only traces of 5-FU were left behind in some samples. This observation is consistent with the in vitro release study which showed that most of the drug was released by one week (Fig. 7).

**Discussion**

Preliminary studies have shown that 5-FU may be efficacious in improving the success rate of glaucoma filtration surgery in patients who are at increased risk of failure. When 5-FU is administered by frequent subconjunctival injection, however, complications may occur.

Different drug delivery vehicles have been tried to deliver 5-FU in such a manner as to avoid frequent subconjunctival injections and to decrease the complication rate. Collagen implants and bioerodible polymers have been used to deliver 5-FU over an extended period of time to a localized area at the site of glaucoma filtration surgery. Cylindrically shaped bioerodible polymers which were inserted into the sclerostomy at the time of glaucoma filtration surgery had an increased number of complications. However, those polymers which were impregnated with 5-FU temporarily prolonged the function of glaucoma filtration surgery as evidenced by lower intraocular pressure, filtration blebs of longer duration and higher outflow facility.

In this study, there were significantly fewer postoperative complications than in the previous study, probably because the polymer shape was less traumatic to the ocular structures. The shape of the polymer probably did not have a mechanical effect on the success rate because there was no difference in intraocular pressure levels between eyes with control polymer versus eyes without polymer following glaucoma filtration surgery.

Also in this study, the intraocular pressures were lower in the experimental eyes for 12 days, which was longer than in the previous study in which 10% 5-FU bioerodible polymers were used. This improvement in the duration of lowered intraocular pressure in the experimental eyes is probably due to the higher concentration of 5-FU in the polymer and longer duration of release. However, it is noted that eventually both experimental and control eyes in this study returned to preoperative intraocular pressure levels. No tonographic measurements were performed in this study; however, based on the results of the previous study, the lower intraocular pressure in experimental eyes was probably due to an increase in outflow facility rather than a decrease in aqueous humor production. This is supported by the histopathological finding at 2 weeks after surgery of an open sclerostomy in the experimental eyes. No abnormal morphological changes were seen in the ciliary body epithelium and trabecular meshwork of 5-FU treated eyes when compared to control eyes by light microscopy.

In summary, this study further confirms the hypothesis that a localized and controlled release of an antifibroblastic agent to the sclerostomy site is beneficial to glaucoma filtration surgery. Further refinements in this new drug delivery system are needed. It is speculated that the most significant factors are the release characteristics and the type of antifibroblastic agents. Work is in progress to optimize these two aspects. From the in vitro release studies, it is strongly suspected that the onset of bleb failure coincides with the exhaustion of the drug. To prolong the duration of drug release, a more hydrophobic polymeric carrier may be required to slow down the diffusion of hydrophilic 5-FU. However, the use of a hydrophobic carrier conflicts with the desire to have an implant disappearing soon after the exhaustion of the drug supply. The use of other antifibroblastic agents may alleviate the delivery problem if the drug is less hydrophilic. The potential of using this polymeric controlled delivery technology in enhancing the success of glaucoma filtration surgery looks promising.

**Key words:** 5-fluorouracil, experimental glaucoma surgery, bioerodible polymers, rabbits, filtering bleb, wound healing.

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**Fig. 7.** A graph of in vitro drug release kinetics of 5-FU from bioerodible polymers. Most of the drug was released by 1 week.
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