Intraoperative 5-Fluorouracil for Filtration Surgery in the Rabbit

J. William Doyle,* Mark B. Sherwood,* Peng T. Khaw,† Susan McGrory,* and M. Fran Smith*

Purpose. Postoperative subconjunctival injections of 5-fluorouracil (5-FU) improve the success rate of filtration surgery, but there is still a 20% or greater failure rate at 1 year in pseudophakic and repeat trabeculectomy cases. The injections are inconvenient to give and may produce corneal epithelial toxicity and other side effects. An alternative method for administration of 5-FU is investigated.

Methods. A masked, randomized, prospective study was undertaken to compare bleb survival and complications in a rabbit model, after either a single intraoperative exposure to 5-FU (50 mg/ml concentration for 5 minutes) or five postoperative injections of 5-FU (5 mg injection on postoperative days 1, 3, 5, 7, and 9), or a combination of both, with controls that received only a 5-minute exposure to distilled water, intraoperatively.

Results. For the control eyes, all blebs were "flat" to masked grading, and intraocular pressure returned to preoperative levels by postoperative day 11. In the group receiving postoperative injections of 5-FU only, blebs were flat and the pressure normalized by postoperative day 14. Rabbits receiving intraoperative 5-FU only, or combined intraoperative and postoperative 5-FU, had consistently higher blebs than the former two groups, and some blebs survived until postoperative day 25. The mean intraocular pressure remained depressed in these groups until postoperative day 21 (P < 0.05 for days 5 through 18). A combination of intraoperative and postoperative 5-FU produced significantly higher blebs than intraoperative 5-FU alone on days 7 to 14.

Conclusions. Intraoperative application of high-dose 5-FU to the filtration site either as a single 5-minute treatment, or in conjunction with postoperative injections of 5-FU greatly prolongs bleb function after filtration surgery in the rabbit. Invest Ophthalmol Vis Sci. 1993; 34:3313–3319.

Trabeculectomy failure is most commonly due to fibrosis and scarring of the bleb at the episcleral level.1,2 Postoperative injections of 5-fluorouracil (5-FU) improve the success rate of filtration surgery in patients with refractory glaucomas,3-10 and more recently have been demonstrated to be beneficial for patients undergoing primary filtration surgery.11,12 Initial clinical trials with 5-FU entailed administering subconjunctival injections twice daily for 1 week, followed by once a day for the second week.4,8 Adverse side effects such as corneal epithelial defects and conjunctival wound leaks were common.4,8 Similar success rates with reduced side effects have been achieved with lower dose regimens.5,7 Despite the decreased dosage, the subconjunctival injections are still uncomfortable for the patient and inconvenient for the physician to administer. Topical 5-FU has been tried, but showed excessive corneal toxicity.13 Various slow-release preparations have been investigated, but all have been associated with localized inflammatory reactions.14-17

The optimal timing, duration, and method of delivery of 5-FU is still uncertain. Recent cell culture work has shown prolonged effects on fibroblast proliferation from a single, 5-minute exposure to 5-FU.18 Based on these data, we have investigated the effect on bleb function of the intraoperative use of 5-FU as a single application, and as an adjunct to postoperative subconjunctival 5-FU injections in the rabbit model.
MATERIALS AND METHODS

A prospective, randomized, placebo-controlled, masked observer study was performed using New Zealand white albino rabbits weighing between 2 and 4 kg. Prior approval of the protocol was obtained from the University of Florida Institutional Animal Care and Use Committee. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

A posterior lip sclerectomy was performed on the right eye of each rabbit by the same surgeon. General anesthesia was induced with an intramuscular injection of ketamine 50 mg/kg and xylazine 10 mg/kg. A partial thickness 8/0 silk corneal traction suture was placed and the eye was pulled downward. A superiorly placed limbal-based conjunctival flap was then raised. Any animal with a conjunctival buttonhole was excluded before randomization and the surgery terminated. The remaining rabbits were randomly allocated to one of four treatment groups: group 1 (6 rabbits) was a control group and received intraoperative distilled water only; group 2 (6 rabbits) received intraoperative 5-FU only; group 3 (6 rabbits) received intraoperative distilled water and postoperative 5-FU injections; group 4 (5 rabbits, 1 rabbit died on postoperative day [POD] 4 secondary to pneumonia) received both intraoperative 5-FU and postoperative 5-FU injections.

Intraoperative drug application was by a sponge (4 x 1 mm dry section of Weck cell sponge) soaked in either distilled water or 5-FU (dissolved in distilled water) at a concentration of 50 mg/ml. The sponge was placed between the conjunctiva and sclera, over the planned filtration site, for 1 minute and then, in hopes of maximizing tissue exposure to the drug, this was repeated four times with freshly soaked sponges for a total of 5 minutes of tissue exposure. The treated area was then thoroughly irrigated with 30 ml of balanced salt solution (Alcon, Ft. Worth, TX) before entering the eye.

A limbal incision 2 mm long was made and the anterior chamber was entered. A 1.5 mm Gass scleral punch was then used to remove standard-sized block of tissue from the posterior edge of the incision area. A peripheral iridectomy was performed through the sclerostomy. The conjunctival incision was then closed with a running 8/0 polygalactin (Vicryl, Ethicon, Somerville, NJ) suture on a B/V 130-4 needle (Ethicon). A 30-gauge paracentesis was performed and balanced salt solution was injected into the anterior chamber to confirm the presence of a patent sclerostomy and inflate a bleb. The conjunctival incision and bleb were inspected to ensure there was no leakage. One drop of atropine sulfate 1% and dexamethasone and neomycin (Maxitrol, Alcon) ointment were instilled at the end of surgery.

For half of the rabbits, subconjunctival injections of 5 mg of 5-FU (Hoffman LaRoche, Nutley, NJ) were given by the nonmasked surgeon on PODs 1, 3, 5, 7, and 9 after the surgery, into the inferior fornix, 180° from the sclerostomy. The injections were 0.1 ml from the 50 mg/ml stock solution, and were given under ketamine anesthesia.

All postoperative observations were made by a masked observer. This observer was another physician, who had previously performed filtration surgeries on rabbits, but was unaware which study group the individual rabbit had been assigned. Intraocular pressure (IOP) was measured, the bleb appearance graded, and corneal staining with fluorescein noted. These observations were made on PODs 1, 3, 5, and 7 before the ketamine anesthesia, and then twice weekly until day 30. Pressure readings were obtained using a Tonopen tonometer (Mentor, Norwell, MA), after anesthetizing the cornea with 1% proparacaine solution. The lids were gently held open without the use of a speculum, and care was taken not to press on the globe. The IOP measurement was repeated, and, to remove any observer bias, the reading recorded was determined as the mean of the first two readings within 2 mm Hg of each other that achieved the 5% confidence level. Bleb size was graded as not present (0); low (1) if there was minimal elevation, but no cystic changes; moderate (2); or high (3) depending on the degree of elevation, avascularity and cystic changes. Corneal staining, using a cobalt light, was noted as present or absent. In rabbits receiving injections, pressure measurements and bleb and corneal assessment were made before anesthesia with ketamine. No histopathologic data were obtained.

Statistical Analysis

Statistical analyses were performed to determine if responses differed between the control and treated groups. Primary endpoints for analysis include IOP, time to IOP failure, bleb appearance, time to bleb failure, and complication rates. For all analyses, a P value less than 0.05 was considered statistically significant. Fisher’s exact test was used to determine if the complication rate for rabbits receiving 5-FU postoperatively differed from those not receiving it postoperatively.

To evaluate differences in IOP between the four groups, analysis of variance was used. Note that assumptions underlying repeated-measures analysis of variance were not satisfied by these data. One-way analysis of variance was performed at each time point to determine if statistically significant differences existed between the groups. If a difference was observed, Fisher’s LSD 0.05 procedure was used to deter-
mine which groups differed. Bleb appearance was analyzed in a similar fashion, except Kruskal-Wallis and rank sum tests were performed because of the ordinal nature of these data.

Survival analyses were carried out for IOP failure and bleb failure. The IOP failure date was defined as the date at which IOP equaled or surpassed the baseline value. The date that bleb appearance was first coded as zero was used as the bleb failure date. Log-rank tests were used to test for overall survival differences among the four groups. If differences were detected, pairwise tests were used to determine which groups differed. Kaplan-Meier survival plots illustrate these data.

RESULTS

Bleb Appearance (Fig. 1)

Group 1: Control. In the control eyes treated with distilled water, the median bleb appearance was graded as zero by POD 7, and all the blebs were flat by day 14. No distilled-water-treated bleb was ever graded as "high."

Group 2: Postoperative 5-FU Injections. In the eyes receiving 5-FU injections only (5 mg on PODs 1, 3, 5, 7, and 9), the median bleb appearance was graded as zero by POD 11 and all the blebs were again flat by POD 14. There was no significant difference in bleb grading or bleb survival comparing the postoperative 5-FU injection only group to the control group.

Group 3: Intraoperative 5-FU. The bleb grading in the eyes treated with intraoperative 5-FU (50 mg/ml concentration for 5 minutes) was higher than that in the control group or the postoperative 5-FU injection only group, but at no day was the difference significant between the groups. The median bleb grading was zero by POD 18 and all blebs were flat by POD 25.

Group 4: Combined Intraoperative and Postoperative 5-FU. In eyes receiving 5-FU both intraoperatively (50 mg/ml concentration for 5 minutes) and postoperatively (5 mg/injection, PODs 1, 3, 5, 7, and 9), the bleb grading was statistically different from control and postoperative 5-FU injection only groups (Groups A and B) on PODs 5, 7, 11, and 14. The blebs in this group were consistently graded as higher than those of the above two groups on all other days, but did not reach levels of statistical significance. The median bleb grading was zero by POD 21, and all blebs were flat by POD 25.

Survival Curves. Bleb survival curves for the four groups are displayed in Figure 2. The failure date for each animal for an endpoint was the first POD on
which bleb appearance was graded as "0 (none)". Significant differences exist between the groups (log-rank = 17.808, \( P = 0.0005 \)). Pairwise differences were indicated between all groups (\( P < 0.02 \)) except control (Group A) and postoperative injections only (Group B), and between intraoperative 5-FU (Group C) and combined intra- and postoperative 5-FU (Group D). All blebs in the combination group survived until POD 18, and some remained until POD 25. All the eyes that did not receive intraoperative 5-FU had flat blebs by POD 14.

**IOP (Fig. 3)**

**Group 1: Control.** In the control eyes, the mean IOP returned to preoperative levels by POD 11.

**Group 2: Postoperative 5-FU Injections.** The IOP in the eyes treated only with postoperative injections of 5-FU returned to preoperative levels by POD 15. There was no statistical difference between the control eyes and the postoperative 5-FU injection eyes at any time.

**Group 3: Intraoperative 5-FU.** The eyes treated with intraoperative 5-FU had decreased IOPs until POD 21. The IOP was statistically different from control eyes on PODs 5, 11, 14, and 18.

**Group D: Combined Intraoperative and Postoperative 5-FU.** The mean IOP of the eyes receiving 5-FU both intraoperatively and postoperatively also returned to preoperative levels by 21 days. The pressure was significantly lower than the control group eyes on PODs 7 to 18, and the postoperative injection only group eyes on PODs 5, 11, and 18.

**Survival Curve.** Kaplan Meier survival plot of the reduction of IOP from baseline is shown in Figure 4. Significant differences were found between the IOP survival curves (log-rank = 9.642, \( P = 0.022 \)). Pairwise differences were indicated between control (Group A) and postoperative injection (Group B) compared to intraoperative 5-FU alone (Group C) and combined intraoperative and postoperative 5-FU (Group D; \( P < 0.03 \) for both comparisons). Eyes treated with combined intraoperative and postoperative 5-FU had the largest decrease in IOP when compared to baseline. All eyes in this group had reduced IOP through postoperative day 18, and two eyes had lowered IOP through day 25. More than 50% of eyes receiving intraoperative 5-FU (Groups C and D) still had reduced IOP compared to baseline at POD 21, whereas in the control group (Group A) and the postoperative 5-FU injection alone group (Group B), 50% of the eyes had returned to baseline IOP by POD 11.
Complications

There was corneal staining with fluorescein in 3 of the 11 eyes that received postoperative 5-FU injections (Groups B and D), but no detectable corneal staining in any of the control or intraoperative 5-FU only treated eyes (Groups A and C). However, this difference did not reach the level of significance. The blebs receiving the intraoperative 5-FU (Groups C and D) were noticeably more avascular than those that did not receive 5-FU intraoperatively. There were no detected buttonholes or incision leaks noted in any animal.

DISCUSSION

The wound healing response in both rabbits and monkeys is known for its vigor and completeness as compared to humans, with uniform closure of routine sclerostomies by POD 14. Antimetabolites have been shown to improve success of glaucoma surgery in humans and in owl monkeys. This study demonstrates in the rabbit model that exposure of tissues at the filtering site to 5-FU for 5 minutes at the time of trabeculectomy surgery delays bleb failure. The intraoperative, single-dose delivery is significantly more effective as compared to alternate day, subconjunctival 5-FU injections alone. When postoperative 5-FU injections are used in conjunction with intraoperative administration, there appears to be further enhancement of bleb longevity.

Intraoperative, single-dose application of the antimetabolite mitomycin C has been reported in human and animal models. To our knowledge, this method of delivering 5-FU has not been published. Direct application of an antimetabolite at surgery has several advantages over postoperative, subconjunctival injections including: delivery to the tissues at the very start of scarring process; the direct delivery to the desired tissues at high concentration; and minimization of the exposure of other tissues to the drug, such as the corneal epithelium. Various slow-release delivery techniques have been tested, including liposomes, collagen implants, collagen shields, or bioerodible polymers. The prolongation of the anti-scarring effect using these systems may be offset by increased complications such as extrusion of the implant, foreign body reactions to the implant material, corneal or systemic toxicity.

Recent cell culture studies have shown that a single exposure of 5-FU to fibroblasts in cell culture result in a prolonged depression in their growth. However, when these fibroblasts recover, their structure and their ability to respond to a growth stimulus appear normal. A single exposure of fibroblasts in cell
culture to mitomycin C demonstrates more marked growth inhibition, and subsequent growth, if observed, is associated with extensive histologic deformity of the cells. This may explain why the intraoperative use of mitomycin C has resulted in very thin blebs and low pressure, because the effects of a single dose of this drug on the fibroblasts may be long-term or permanent. In some mitomycin-treated human eyes, late bleb leaks and hypotony associated with thin atrophic blebs have resulted in visual reduction. Because fibroblast growth appears delayed, but otherwise normal in cultures treated with 5-FU, it may be possible to obtain a temporary and more titratable decrease in wound healing with this drug. This would inhibit fibroblast proliferation in the immediate postoperative period, but would not prevent later proliferation to maintain bleb integrity. The rabbit as a surgical model most closely resembles the healing responses seen in difficult refractory glaucomas in humans. Bergstrom et al demonstrated considerable increase in bleb longevity as well as IOP lowering (mean duration 60 days) in the rabbit model when intraoperative mitomycin C was used. To our knowledge, the effects of postoperative injections of 5-FU on bleb function after filtration surgery in the rabbit has not been investigated. In our study, alternate-day, postoperative injections were chosen to resemble the postoperative course of a primary trabeculectomy in humans. When used alone this regimen only slightly prolonged bleb survival in the aggressively healing rabbit model but the effect of postoperative 5-FU therapy might be enhanced by increasing the number of injections given. When 5-FU was applied intraoperatively, directly to the sclerotomy site, bleb survival was significantly increased. A combination of intraoperative and postoperative administration of 5-FU produced the most marked prolongation of bleb function.

The addition of 5-FU intraoperatively, either by 5 1-minute exposures or by 1 5-minute exposure, may greatly enhance the effectiveness of this drug as an anti-scarring agent in filtration surgery. Clinically, 5-FU may have advantages over mitomycin C. The treatment effect of 5-FU demonstrated in cell culture appears to be of shorter duration, and the long-term integrity of the conjunctiva over the bleb may thus be less compromised with 5-FU administration than after mitomycin C usage. Also, for those patients in whom low-dose, postoperative injections of 5-FU are indicated but in whom it would be difficult to administer (i.e., children, mentally compromised patients, those
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traveling long distances, and others), the use of a single, intraoperative dose of 5-FU may offer an acceptable antimetabolite therapy to enhance bleb function. Further work to determine the optimum concentration and duration of exposure to 5-FU to maximally enhance bleb function must be done.

Key Words

glaucoma, antimetabolite, surgery, 5-fluorouracil, rabbit, filtration surgery

References