Subconjunctival Sustained Release 5-Fluorouracil

David L. Blandford, Thomas J. Smith, Joel D. Brown, P. Andrew Pearson, and Paul Ashton

The authors have developed a sustained release device for 5-fluorouracil (5-FU) made up of a 12 mg pellet of drug coated in a mixture of permeable and impermeable polymers. When implanted subconjunctivally in rabbits, these devices released 5-FU at approximately 1 mg/d for over 10 days. Devices were implanted into four cynomolgus monkey eyes after posterior lip sclerotomy. One eye (treatment) received a device that contained 12 mg 5-FU and the other eye (control) received a placebo device that contained no drug. In control eyes, intraocular pressures returned to normal within 1 wk. In treatment eyes, pressures remained significantly lower throughout the experimental period (3 mo). There was no indication of impaired wound healing, corneal toxicity, inflammation, or damage to the ciliary body in rabbits or monkeys.

Filtering surgery in glaucoma patients creates an alternative aqueous pathway from the anterior chamber to the subconjunctival space. The overall success rate for these operations varies from series to series but is approximately 85%. However, in young, black, or aphakic patients or in those who have had a previous failed filter, the success rate may be as low as 20%. Failure usually is due to the proliferation of fibroblasts, which leads to scarring and subsequent blockage of the filter. Successful filtration in these patients primarily depends on a suppression of fibroblast proliferation.

Numerous attempts have been made to pharmacologically inhibit fibroblast proliferation and hence improve the success rate of filtering surgery. Proliferation of fibroblasts can be reduced in culture by administering low levels of 5-fluorouracil (5-FU; 50% inhibition at 0.2 µg/ml). In rabbits, twice daily subconjunctival (SC) injections of 5-FU or drops four times a day maintain levels above 0.2 µg/ml in the aqueous and vitreous and effectively reduce proliferation. SC injections of 5-FU have been demonstrated to be clinically valuable in glaucoma patients with a poor prognosis. However, SC injections and topical drops cause extremely high peak levels of drug, especially in the cornea and conjunctiva (over 500 µg/g) and are accompanied by a high incidence of side effects, primarily involving the corneal epithelium and the conjunctival wound. The frequent SC injections and the prolonged hospitalization often necessary are additional serious drawbacks to this therapy.

It is uncertain what, if any, intraocular 5-FU levels need be attained, because the principal site of this drug's therapeutic action is in the SC space. We have developed an implantable, sustained release device for 5-FU that can be implanted SC. This may allow low, therapeutic drug levels to be maintained at the site of action without exposing the rest of the eye to the high toxic levels obtained with SC injections.

Materials and Methods

Materials

5-fluorouracil was obtained from Sigma Chemical Company (St. Louis, MO). NaH₂PO₄ and Na₂HPO₄ were purchased from Aldrich Chemical Company (Milwaukee, WI), as was polyvinyl alcohol (PVA; 98% hydrolyzed, 76,000-78,000 molecular weight). Ethylene vinyl acetate (EVA, grade) was obtained from Du Pont (Wilmington, DE).

Device Construction

EVA membranes were prepared by compressing 3.5 g of EVA under 4 metric tons at 190°C into sheets 0.6 mm thick. Twelve milligram pellets of 5-FU were prepared in a 2.5 mm tablet die. These pellets were coated in a 10% (weight/volume) PVA solution and allowed to dry. They then were coated on three sides with a sheet of EVA, and a circular disc of EVA (precoated in 10% PVA) was fixed onto the fourth side. After they were coated again with 10% PVA and allowed to dry, suture tags of PVA were attached and
the entire assembly was heated at 180°C for 4.75 hr (Fig. 1).

In Vitro Release Rate

Devices were immersed in 5 ml of a serum solution at 37°C. This solution was composed of 80% calf serum and 20% phosphate buffer (0.05 mol/l, pH 7.4) and contained 0.01% thimerosal as a preservative. One hundred-microliter samples were periodically removed for analysis. One hundred microliters of 0.01 N HCl and 200 μL of acetonitrile were added to each sample. After vortexing, samples were allowed to stand for 60 min at 5°C before being centrifuged at 18,000 rpm for 30 min. Three hundred microliters of the supernatant then was removed and freeze dried. The products then were rehydrated with 75 μL 0.1N HCl and injected onto a reverse phase C-18 Axxion (Aldrich Chemical Co.) high performance liquid chromatography (HPLC) column (5 μm x 25 cm). The mobile phase was 0.01% ammonium acetate buffer, pH 4.0, and the flow rate was 1.0 ml/min. Detection was by ultraviolet light at 266 nm.

Release Rate in the Rabbit

All animal work was carried out in accordance with the ARVO Resolution on the Use of Animals in Research. The release of 5-FU from the devices was measured in vivo in 14 New Zealand White rabbits (1.5–2 kg). Animals were anesthetized with xylazine (15 mg/kg), ketamine (40 mg/kg), and topical proparacaine (0.5%). Devices were sterilized in ethylene oxide by the sterile products division of the Veterans Administration Hospital, Lexington, Kentucky. A 1.5 cm incision through conjunctiva and Tenon’s capsule was made superiorly, parallel to the limbus. Bare sclera was exposed by blunt dissection. A device was sutured to bare sclera of each eye 1 cm from the limbus. The Tenon’s capsule and conjunctiva then were closed. Gentamicin drops and erythromycin ointment were instilled into the eye. All animals recovered without complications. Two rabbits were killed 1, 4, 7, 9, 11, 14, and 21 days after implantation, and the implants were removed. Recovered devices were cut in half and immersed in 25 ml phosphate buffer (pH 7.4). Sonication for 90 min ensured complete dissolution of 5-FU. The concentration of 5-FU then was determined by HPLC analysis, and the amount of drug remaining in each device was calculated.

Toxicology Studies in the Rabbit

Devices were implanted SC into the eyes of four rabbits as described above. Baseline electroretinograms, intraocular pressures, and fundus photos were obtained before and 6 wk after implantation. Animals were killed by pentobarbital injection, and eyes were examined histopathologically.

Effect of Device Implantation on Maintenance of Filters in the Monkey

Four cynomolgus monkeys (3–4 kg) were used in this study. After they had fasted for 12 hr, the animals received atropine (0.05 mg/kg), ketamine (10 mg/kg), and xylazine (2 mg/kg). General anesthesia was induced with halothane (100 ml/min) until the animal became unresponsive to touch stimuli. Thereafter, it was adjusted as needed. A pediatric lid speculum was inserted and a 7-0 silk traction suture was placed superiorly at the corneolimbal junction to infraduct the eye. A limbus-based flap was created by making a 1.5 cm incision through conjunctiva and Tenon’s capsule approximately 1 cm posterior to and parallel with the limbus. The anterior chamber was entered using a 75 Beaver blade (Rudolph Beaver Inc., Waltham, MA). Then, a 1 mm corneoscleral punch was used to form a full thickness posterior lip sclerectomy. Tenon’s capsule and conjunctiva were reaproximated using a running two-layer closure with 7-0 silk. A SC injection of 4 mg of triamcinolone was given 180° from the filtration site; then, gentamicin drops and erythromycin ointment were instilled. All animals recovered without complication.
Intraocular pressure (IOP) readings were performed at weekly intervals postoperatively with a Digi-
lab (Cambridge, MA) pneumotonometer. Four weeks postoperatively the first animal was killed. The ani-
mal was fixed by exsanguination/perfusion using 2.5% glutaraldehyde. The orbital contents were re-
moved through a posterior approach to cause as little disturbance to the tissues as possible. The eyes then
were prepared for histologic examination by light mi-
croscopy. Three months postoperatively the remain-
ing animals were killed and their eyes were obtained
in the same manner.

Results

In Vitro Release Studies

Release from the devices was found to be pseudo
zero order, with more than 80% of the drug released in
10 days (Fig. 2A).

Release Rate in the Rabbit

There was no evidence of device-related toxicity
(corneal epithelial defects, hemorrhage, or poor wond healing) in treatment or control eyes during
this study. Release of 5-FU from the devices was
found to be pseudo zero order; all of the 5-FU was
released within 14 days (Fig. 2B).

![Graph](https://via.placeholder.com/150)

**Fig. 2.** (A) In vitro release of 5-fluorouracil (5-FU) from the de-
vice into serum at 37°C and pH 7.4. Standard deviations are indi-
cated (n = 6). (B) Amount of 5-FU remaining in the device after
subconjunctival implantation. X axis represents the number of
days devices were left in the subconjunctival space before analysis.
Standard deviations are indicated (n = 4).

Toxicology Studies in the Rabbit

All rabbits recovered from the procedure without
complications and showed no indications of discom-
fort. There was no corneal clouding, infection, wound
dehiscence, hemorrhage, chemosis, or epithelial de-
fects at any time during the experiment. There was
moderate inflammation of the conjunctiva after im-
plantation, which resolved after the first week. IOP
readings during the experiment showed no significant
difference between implanted and control eyes. Fundus examinations and electroretinograms were un-
changed from baseline. Histology by light microscopy
showed no abnormalities. A thin membrane of con-
nective tissue (3-5 cells thick) was seen surrounding
the implant, with more reaction around the suture
than the device (Fig. 3).

Effect of Device Implantation on Maintenance of
Filters in the Monkey

Pressures in control eyes after filter operations ini-
tially were low but quickly returned to normal. Hypo-
tonia was seen in only two control eyes after 1 wk
(mean IOP was not statistically different from control
after 4 days). In treatment eyes, pressures remained
low (less than 10 mmHg) throughout the study in all
animals (Fig. 4). The difference in pressure between
treatment and control eyes was statistically significant
at all time points after 4 days (P < 0.01). Histologic
examination of the control eyes obtained after 4 wk or
3 mo showed no evidence of stoma or bleb. Filtration
blebs were identified in all treatment eyes. In the ani-
mal killed after 4 wk, a stoma with a tract back to the
implant (consistent with a thick walled bleb) was
identified. All treatment eyes of animals killed after 3
mo showed filtration blebs (two thin walled and one
thick walled) and one open stoma was identified. In
all treatment eyes, numerous vacuoles were found
under the conjunctiva above the tract; this was not
seen in control eyes (Fig. 5).

There was no evidence of wound dehiscence, cor-
neal cloudiness, or epithelial abnormalities. Fundus-
scopic examination revealed no evidence of choroidal
effusion, hemorrhage, or retinal detachment in
treated or control eyes, although the optic nerve head
appeared to be swollen in treated eyes.

Histologic examination showed no evidence of an
inflammatory reaction to the device in the 4 wk or 3
mo eyes. The ciliary body, retina, and cornea ap-
peared normal in all animals. However, in the animal
killed after 4 wk a small area of the retina immediately
posterior to the implantation site appeared to contain
vacuoles. This was not observed in control eyes or in
treatment eyes obtained after 3 mo.
Discussion

Several attempts have been made to develop implantable, sustained release devices of 5-FU for SC use. A sustained release form of 5-FU can be expected to prevent the peak tissue levels obtained with SC injections. Another potential advantage with this mode of administration is that 5-FU would be delivered at the required site of action, further reducing the possibility of toxic side effects. In 1987, Lee and coworkers investigated the use of a bioerodible polyanhydride matrix containing 10% 5-FU. In rabbit eyes that received these devices, pressures remained lower than in controls for approximately 2 wk. Subsequent work showed that little sustained release was achieved from these devices, with over 60% of the 5-FU released in the first 2 days. The polyanhydride used (poly-[bis9p-carboxyphenoxy)propane-sebacicacid]copolymer), has since been shown by Tamargo and coworkers to induce an acute inflammatory reaction in the rat brain. Devices made from the same polyanhydride have been implanted SC to deliver 5-fluoro-

Fig. 3. Section of device 6 wk after subconjunctival implantation in the rabbit. Note the thin layer of cells over the device wall (brown) and the lack of inflammation.

Fig. 4. Intraocular pressures of monkeys after trabeculectomy. Squares represent animals that received sustained release 5-fluorouracil device. Triangles represent control animals that received placebo device. Mean values are plotted and standard deviations are indicated. For time points up to 4 wk, n = 4; for all subsequent time points, n = 3. Difference between treatment and control animals was significant (P < 0.01) for all time points after 4 days.

Fig. 5. Section of monkey eye 3 mo after trabeculectomy and 5-fluorouracil device implantation. B, bleb. C, cornea. CB, ciliary body. D, device. L, lid. S, sclera. *, no inflammation around the device. **, significant inflammation around silk suture.
5-fluorouridine is approximately 100 times more potent than 5-FU. In the present study, devices extended the duration of success of filtration surgery in monkeys from 8.5 ± 4.0 days to 26.0 ± 9.2 days. Significant local toxicity (conjunctival wound leak and impaired wound healing) was observed, and, in concurrence with the work of Tamargo, there was some evidence of inflammation. The devices also gave rapid release of 5-fluorouridine (approximately 50% in 2 days). Liposomal injections of 5-fluorouridine also have been tested in monkeys but were accompanied by unacceptable local and systemic toxicity, possibly because of the large doses of drug used (over 20 mg).

The use of 5-FU collagen implants also has been investigated, but this polymer was found to elicit a severe inflammatory response. An inflammatory response to the device itself is highly undesirable because this may result in enhanced scarring and accelerated filter failure.

The devices used in the present study gave sustained release of 5-FU (1 mg/day) in vivo. These devices, however, are composed of EVA and PVA. These polymers are not bioerodible, so the devices, like Moltino tubes, would remain implanted in the eye indefinitely. Although this is not ideal, the devices produced no indication of bio-incompatibility in rabbits or monkeys, and previous reports indicated that PVA is biologically inert in humans. In our own experience, devices made up of PVA and EVA (designed to deliver ganciclovir intravitreally) caused no inflammation or other bio-incompatibility when implanted intravitreally into human eyes.

The cynomolgus monkey is considered a good model for glaucoma because the anatomy of the outflow system is similar to that of humans. Glaucoma filtering operations performed in this species uniformly fail because of exuberant proliferation of SC fibroblasts. Thus, the cynomolgus monkey is an excellent model for high-risk glaucoma patients.

In the present study, there was a prolonged reduction of IOP in monkeys that received 5-FU implants. The lower IOPs appeared to be the result of a functioning filter because there was no evidence of ciliary body toxicity, and stomas or blebs were identified in all treatment eyes. Although 5-FU was released from the devices within 2 wk, IOPs remained low for 3 mo. This suggests that if proliferation can be controlled during the initial postoperative period (during the most intense trauma induced inflammation) filters may remain open indefinitely. This also is implied for SC injections of 5-FU that are given for up to 2 wk in high-risk patients.

There was no evidence of corneal toxicity or wound abnormalities, suggesting that sustained release SC implantation of 5-FU may avoid the toxicities normally associated with this agent. Histopathologically, there was no evidence of an inflammatory response to the device itself. The swollen optic nerve head observed can be attributed to prolonged hypotonia in the treatment eyes. The retinal vacuoles seen after 4 wk may have been the result of drug-related toxicity. However, because this is one observation, interpretation is difficult. Further work should investigate this more closely.

In this animal model, filters uniformly fail. Maintenance of a functioning filter in the present study suggests that this drug delivery system may be useful in high-risk patients. In humans, the lower intrinsic fibroblastic proliferation and the concurrent use of steroids could be expected to further improve outcome. A phase one clinical trial is underway.

Key words: filtration, 5-fluorouracil, glaucoma, subconjunctival sustained release.

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