Glucoma Filtration Surgery in Nonhuman Primates Using Taxol and Etoposide in Polyanhydride Carriers

Henry D. Jampel,* Diane Thibault,* Kam W. Leong,† Paroo Uppal,† and Harry A. Quigley*

Purpose. To determine the effect of taxol and etoposide, hydrophobic drugs with antifibrosis activity, on the outcome of filtration surgery in glaucomatous monkeys.

Methods. Elevated intraocular pressure was produced bilaterally in eight cynomolgus monkeys by laser treatment of the trabecular meshwork. Four animals subconjunctivally received a polyanhydride disk containing 1 mg etoposide at the time of posterior lip sclerectomy in one eye; the other eye received an identical disk without drug. Similarly, four animals received a disk containing 50 μg of taxol in one eye and a blank disk in the other.

Results. Eyes treated with taxol had lower intraocular pressures than control eyes from 20 days after surgery until death. Eyes with satisfactory filtration bleb appearance and patent fistulae on histologic examination had lower intraocular pressures. The intraocular pressure was lower and the duration of success longer in the etoposide-treated eyes (mean, 16 days) compared to that of the fellow eyes (mean, 10 days), but the difference was not statistically significant.

Conclusions. Use of polyanhydride disks containing taxol, but not etoposide, had a marked beneficial effect on intraocular pressure and bleb appearance after experimental filtration surgery in monkeys. The difference between the two agents may result from the greater anti-proliferative potency of taxol and its greater duration of release from the polymer.


The principal cause of the failure of glaucoma filtration surgery is scarring in the subconjunctival space.1-2 Agents that interfere with postoperative wound healing improve the success rate of glaucoma filtration surgery both in experimental animals and in patients. Drugs with proven efficacy in humans include topical corticosteroids,3,4 fluorouracil,5 and mitomycin C.6-7 However, subconjunctival injections of fluorouracil8,9 and topical application of mitomycin C10,11 are associated with adverse side effects, some of which are vision-threatening. Furthermore, glaucoma filtration surgery fails in a substantial minority of high-risk eyes despite the use of these agents.

Several investigators have explored the feasibility of employing a subconjunctivally implanted controlled-release carrier for drugs at the time of glaucoma filtration surgery.12-18 Such systems may provide a more effective and safer way of improving the success rate of glaucoma filtration surgery. We found that 5-fluorouridine delivered by polyanhydride carrier19 improved the success of experimental glaucoma surgery modestly. There was, however, an undesirable rapid release of the drug from the polymer. We chose to test etoposide and taxol because the release kinetics of hydrophobic agents might be more favorable. Based on encouraging in vitro data,20 we have now evaluated the efficacy of these agents in an experimental model of glaucoma filtration surgery.

MATERIALS AND METHODS

Preparation of Bioerodible Polyanhydride Copolymers

The drug carriers used in the experiments consisted of the co-polyanhydries of 1,3-bis(p-carboxyphenyenoxy)
propane and sebacic acid. The polymers were synthesized by the melt-polycondensation method and characterized by Fourier-transformed infrared spectroscopy and gel permeation chromatography. One milligram of etoposide (Bristol-Myers Oncology, Evansville, IN) was incorporated into a 25:75 copolymer and 50 μg of taxol (National Cancer Institute, Bethesda, MD) was incorporated into a 30:70 copolymer by compression molding. These proportions of copolymer had previously been determined to yield the best in vitro release kinetics for these two agents. Fifty micrograms of taxol was the maximum amount that was not toxic to the cornea in pilot studies. The amount of etoposide was the maximum amount that could be incorporated into the carrier. The resulting disks were coated on either side with an additional 1 mg of pure copolymer to improve the drug release profile. The finished products were solid disks measuring 3 mm in diameter and 1 mm in thickness, and weighing 10 mg. The disks were stored under desiccation and sterilized by ultraviolet light before use.

**Experimental Glaucoma Filtration Surgery**

Sustained elevation of intraocular pressure (IOP) was achieved in 16 eyes of 8 male cynomolgus monkeys (Macaca fascicularis) weighing approximately 6 kg by argon laser treatment of 360° of the anterior chamber angle at least 1 month before surgery. None of the eyes were clinically inflamed at the time of surgery.

A posterior lip sclerectomy was performed as described previously, with these modifications: 1) no tenonectomy was performed; 2) before creation of the fistula, in masked fashion, a polyanhydride disk, with or without drug, was sewn to the sclera posterior to the site of the fistula with a 8–0 vicryl suture; and 3) Tenon’s capsule and conjunctiva were closed separately with a running locked 8–0 vicryl suture. These maneuvers were performed to minimize the chance of migration or extrusion of the disk.

We instilled erythromycin ophthalmic ointment (Fougera, Melville, NY) at the end of surgery. No antibiotic, cycloplegic, or corticosteroid medication was administered after that time. An experienced animal caretaker assessed the general well-being of the animal daily. Only one eye of an animal was operated on a given day. The animals were never bilaterally blinded (functional) and did not appear to experience more than the minimal discomfort that humans experience after similar surgery. All procedures were approved by the Animal Care Committee of The Johns Hopkins University and were compatible with the Association for Research in Vision and Ophthalmology Resolution on the Care of Animals in Research.

We examined each eye in masked fashion at least every 2 to 3 days after surgery for the first 3 postoperative weeks, and approximately once weekly thereafter. The examinations were performed under intramuscular ketamine hydrochloride (10 mg/kg) sedation, and topical proparacaine hydrochloride anesthesia. They consisted of IOP determination by Goldmann applanation tonometry; slit-lamp examination to assess bleb status, anterior chamber depth, and amount of inflammation; and status of the internal opening by gonioscopy. Bleb appearance was subjectively graded as functional (elevated and pale), or nonfunctional (flat and hyperemic). The internal fistula was graded by gonioscopy as open, possibly open, or closed. Central corneal thickness was measured 1 to 4 weeks preoperatively and 4 to 6 weeks postoperatively using an ultrasonic pachymeter (DGH Technologies, Exton, PA). Each measurement was made four times. Three milliliters of blood was drawn from the femoral vein of three animals in the taxol experiment with a 22-gauge needle both at the time of surgery and 1 to 2 months after surgery.

**Histologic Preparation**

The animals in the etoposide study were killed by exsanguination under deep pentobarbital anesthesia. Fixation was carried out by sequential perfusion in retrograde fashion through the abdominal aorta of 500 ml of physiological saline solution containing 1 g/dl of procaine hydrochloride, followed by approximately 11 of 2.5% glutaraldehyde in cacodylate buffer. A subtotal exenteration of the orbits was immediately performed, preserving the conjunctiva. The globes were then immersed in 4% paraformaldehyde and 2.5% glutaraldehyde at room temperature overnight. Because unfixed retinas were needed for unrelated experiments, the monkeys in the taxol experiment were killed by an overdose of pentobarbital and then subtotal exenterations of the orbits were performed. The anterior segments were immersion-fixed in 4% paraformaldehyde and 2.5% glutaraldehyde.

We prepared 1-mm–wide tissue segments that were oriented perpendicular to the limbal wound, containing both cornea and sclera on either side of the wound. Other tissue sections containing the ciliary processes adjacent to the fistula were taken from each eye. All tissue was washed in 0.05 N sodium cacodylate buffer and postfixed in 1% osmium tetroxide in 0.05 N sodium cacodylate containing 10% sucrose. The segments were serially dehydrated and embedded in epoxy resin. One-micron– thick sections were placed on microscope slides and stained with toluidine blue for 30 seconds. The status of the subconjunctival tissue, internal opening, and ciliary epithelium were evaluated by an observer masked as to whether the specimen came from a drug-treated or control eye.
TABLE 1. Preoperative Parameters

<table>
<thead>
<tr>
<th></th>
<th>Etoposide</th>
<th>Etoposide Controls</th>
<th>Taxol</th>
<th>Taxol Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser Energy (J)</td>
<td>54 ± 18*</td>
<td>52 ± 14</td>
<td>88 ± 22</td>
<td>79 ± 33</td>
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<td>Preoperative IOP (mm Hg)</td>
<td>30.0 ± 6.9</td>
<td>40.3 ± 12.8</td>
<td>42.6 ± 7.9</td>
<td>33.8 ± 4.9</td>
</tr>
</tbody>
</table>

* Mean ± SD.
† Paired t test.
‡ Mean of last three measurements before surgery.

RESULTS

Preoperative Characteristics: Experimental and control eyes were similar in terms of the amount of laser energy used to induce elevation of IOP (Table 1). The eyes that received taxol had significantly higher IOP than their fellow control eyes.

Intraocular Pressure: In all four eyes that received etoposide the IOP remained lower for a longer time after surgery than in their fellow eyes that received polymer without drug (Fig. 1). However, the IOP had returned to > 20 mm Hg by 19 days after surgery in all eyes. This is a criterion for surgical failure that we have previously used. By this criterion,
the last day of surgical success was 16 ± 6 days in the etoposide-treated eyes and 10 ± 3 days in the control eyes \((P = 0.18, \text{paired } t\text{ test})\).

In the taxol experiment, all eyes except one had IOP ≤ 10 mm Hg for the first 15 days after surgery (Fig. 2). From postoperative day 20 onward, the IOP was statistically significantly lower in the eyes receiving taxol. For example, 40 days after surgery the IOP was 3.0 ± 3.2 mm Hg in the taxol-treated eyes and 25.2 ± 14.1 in the control eyes \((P = 0.035)\). Using a criterion of > 20 mm Hg for failure for the operation, three of the four control eyes failed at postoperative days 16, 17, and 21. The fourth control eye (m 946) did not reach an IOP of 20 mm Hg. None of the four taxol-treated eyes failed by this criterion.

**Clinical Findings**

One week postoperatively, two of four etoposide-treated eyes and zero of four control eyes had pale, fluid-filled blebs that could be considered functional. At the same time three of four etoposide-treated and one of four control eyes had a gonioscopically patent internal fistula. Two weeks after surgery only one etoposide-treated eye (m 883) had a clinically apparent bleb and an open fistula; none of the control eyes did.

Four out of four taxol-treated eyes had pale, elevated blebs (Figure 3) for the duration of the postoperative period (110 to 150 days after surgery). Three of the four had unequivocally open fistulas clinically at the time of death. One control eye (m 946) had both an elevated bleb and a patent fistula at the time of death; another control eye (m 951) had a patent fistula and no bleb.

There was no detectable difference in central corneal thickness between drug-treated and control eyes (Table 2). Preoperative and postoperative complete blood counts showed that none of the animals had a decrease in their peripheral blood cell count after surgery.

![Graphs showing intraocular pressure over time](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933170/...)

**FIGURE 2.** Intraocular pressure after surgery in each of four monkeys in the taxol experiment. Intraocular pressure in the eye receiving taxol are represented by the solid line; the fellow eyes that received a blank polymer disk are represented by the dashed lines. The horizontal line at 20 mm Hg represents one criterion of surgical failure that we have used previously.10 By this criterion, none of the four taxol-treated eyes failed; one of the control eyes (m 946) also did not fail. The scale on the vertical axis differs from graph to graph.
Complications (Table 3): One taxol-treated eye (m 947) had a wound leak at the conjunctival incision site from postoperative days 23 to 80 (Table 3). On day 43 a conjunctival epithelial defect over the bleb developed in one control eye (m 946) in the taxol experiment that remained Seidel positive with pressure until death. Neither of these leaks was associated with a shallow anterior chamber. None of the disks eroded through the conjunctiva.

No corneal epithelial defects were noted. However, corneal epithelial pigmentation was present in all four eyes that received taxol, but resolved over time. The appearance was similar to the striate melanokeratosis that has been noted in humans after subconjunctival fluorouracil administration. Superficial corneal vessels developed in two of taxol-treated eyes and extended 3 to 4 mm into the cornea. In four of the five eyes with corneal edema, the edema was noted during the first week after surgery, in association with low IOP, and resolved within 2 days to 2 weeks. In the fifth eye, corneal edema occurred as the IOP rose from 2 mm Hg to 50 mm Hg.

Although shallowing of the anterior chamber occurred postoperatively in two eyes that received taxol and in two control eyes in the taxol experiment, it resolved earlier (on days 7 and 10) in the control eyes than in the taxol-treated eyes (days 36 and 37). No choroidal detachments were seen. Fibrinous anterior chamber reactions and hyphemas occurred in all groups and have been frequent features of glaucoma filtration surgery in monkeys in our hands. Mild cataractous changes were present in three of four taxol-treated eyes and in one of four of the other eyes. All cataracts were mild and occurred at varying locations within the lens.

Histology: All eyes in the etoposide experiment were examined after the operation had failed clinically. Tissue composed primarily of spindle-shaped cells that were densely packed filled the fistula tract from the subconjunctival space to the level of Descemet's membrane in three of four etoposide-treated eyes and three of four control eyes. In two of four etoposide-treated eyes the granulation tissue in the subconjunctival space was less cellular than in the corresponding control eyes. Fragments of the polyanhydride disk were present in the subconjunctival space of both eyes of one animal and were associated with foreign body giant cells.

TABLE 2. Clinical Data

<table>
<thead>
<tr>
<th></th>
<th>Etoposide</th>
<th>Etoposide Control</th>
<th>Taxol</th>
<th>Taxol Control</th>
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<tr>
<td>Pale, Elevated</td>
<td>2/4</td>
<td>0/4</td>
<td>4/4</td>
<td>1/4</td>
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<tr>
<td>Gonioscopically</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open Fistula*</td>
<td>3/4</td>
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<tr>
<td>Preoperative</td>
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<tr>
<td>Pachymetry (µ)</td>
<td>438 ± 16†</td>
<td>449 ± 11</td>
<td>406 ± 22</td>
<td>404 ± 30</td>
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<tr>
<td>Postoperative</td>
<td></td>
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<tr>
<td>Pachymetry (µ)</td>
<td>452 ± 23</td>
<td>447 ± 17</td>
<td>421 ± 62‡</td>
<td>402 ± 38</td>
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</table>

* Evaluated 1 week after surgery (etoposide experiment) or immediately before euthanasia (taxol experiment).
† Mean ± SD.
‡ None of the postoperative pachymetry values were statistically different from the preoperative values.
TABLE 3. Complications

<table>
<thead>
<tr>
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<th>Etoposide</th>
<th>Etoposide Control</th>
<th>Taxol</th>
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</thead>
<tbody>
<tr>
<td>Wound Leak</td>
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<td>Corneal Epithelial Pigmentation</td>
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<td>Corneal Edema</td>
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<tr>
<td>Corneal Endothelial Pigmentation</td>
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<td>0/4</td>
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<tr>
<td>Anterior Chamber Shallowing</td>
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<tr>
<td>Fibrinous Anterior Chamber Reaction</td>
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<tr>
<td>Hyphema</td>
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<tr>
<td>Lens Opacity</td>
<td>1/4</td>
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</table>

In the taxol experiment, none of the fistulae in either the experimental or control eyes were occluded to the level of Descemet's membrane. Two of the taxol-treated eyes had fistulae partially occluded by iris and ciliary body. The subconjunctival space contained only loose granulation tissue (Fig. 4) in the four taxol-treated eyes and the one control eye with a bleb (m 946). There was dense granulation tissue in the other three control eyes (clinical failures). Neither remnants of the polyanhydride disks nor foreign body giant cells were observed. The ciliary processes had a normal epithelial bilayer and stroma in both etoposide- and taxol-treated eyes as well as in the control eyes.

DISCUSSION

The implantation of polyanhydride disks containing 50 μg of taxol promotes the success of glaucoma filtration surgery in glaucomatous monkey eyes. The taxol-treated eyes had lower IOP than their fellow control eyes, and they had filtration blebs and patent fistulas. Although we cannot exclude definitively the possibility that either decreased aqueous humor production or inflammation caused the IOP to be lower, the absence of histologic changes in the ciliary processes and of clinically apparent inflammation makes these alternatives unlikely.

Unlike taxol, etoposide did not have a demonstrable effect on the outcome of glaucoma filtration surgery. With a larger number of animals, a statistically significant effect might have been observed, but certainly would not have been greater than that previously observed with 5-fluorouridine.19 We can speculate that taxol was more effective than etoposide because of its greater potency and its more favorable release kinetics from the polymer.

Taxol incorporated into polyanhydride disks joins a growing list of controlled release systems for drugs that have been evaluated experimentally for use in conjunction with glaucoma filtration surgery. Some of these other systems include collagen sponges containing fluorouracil or bleomycin,12,15 polyanhydrides containing fluorouracil,13 fluorouridine,19 mitomycin,16 and daunorubicin,28 liposomes containing fluorouridine,14 polyvinyl alcohol, and ethylene vinyl acetate membranes containing fluorouracil,17 and poly(lactic acid) microspheres containing doxorubicin (Adriamycin, Adria Laboratories, Columbus, OH).18 Although comparisons between different studies are difficult, polyanhydrides containing taxol appear to be at least as effective as the other systems described. Collagen sponges29 and the polyvinyl alcohol and ethylene vinyl acetate membranes30 have undergone preliminary testing in humans.

The eyes in these experiments, particularly the taxol experiment, behaved differently from eyes that we have previously studied. First, rather than failing 7 to 10 days after surgery, one of the control eyes in the etoposide experiment and three of the control eyes in the taxol experiment did not fail until 3 weeks after surgery. One control eye in the taxol study did not fail.
Second, we observed marked postoperative hypotony in the early postoperative period in both the taxol-treated eyes and their contralateral control eyes. Three of the four control eyes had IOP close to 0 mm Hg until the IOP abruptly rose approximately 20 days after surgery. Inflammation not detectable by slit-lamp examination and decreased aqueous humor production without histologic evidence of ciliary process damage are possible explanations for the hypotony. Other investigators have reported very low IOP after glaucoma filtration surgery in monkeys even in control eyes without blebs or patent fistulas. For instance, in the original article on fluorouracil, the IOP in the control eyes 14 weeks after surgery ranged from 3 to 10 (mean 6 ± 2.5) mm Hg. However, these eyes did not have laser-induced elevated IOP preoperatively.

Our studies, employing small numbers of animals, were not designed specifically to evaluate the toxicity and side effects of the drugs and the controlled release carrier. We already knew that polyanhydrides elicit a mononuclear and giant cell reaction when implanted in the subconjunctival space at the time of glaucoma filtration surgery. Preliminary studies suggested that higher doses of taxol could cause corneal edema. Of particular note in our study was the corneal epithelial pigmentation seen in all eyes that received taxol. This probably represents the pigment migration that has been observed in humans after glaucoma filtration surgery with fluorouracil injections, and we have seen it previously in monkey eyes. The pigment migration was not preceded by an epithelial defect, as is commonly seen in humans. The presence of peripheral corneal neovascularization in two taxol-treated eyes may also be significant. Corneal new vessels have also been observed after glaucoma filtration surgery with mitomycin C in rabbits. The anterior chamber inflammation, shallowing and hyphema, as well as mild cataracts perhaps related to them, are commonly seen after full-thickness glaucoma filtration surgery in monkeys. We did not study the effect of taxol on the retina and optic nerve because the tissue was used in other experiments. In one study, injection of 35 µg taxol intravitreally resulted in optic disc pallor in 25% of rabbit eyes treated.

Taxol is a highly water-insoluble agent derived from the bark of the Western yew tree. Its mechanism of action is unique among antiproliferative drugs, because it promotes microtubule assembly and inhibits microtubule depolymerization. In cell culture, taxol is a potent inhibitor of rabbit chorioretinal fibroblast proliferation and of rabbit Tenon’s capsule migration. Intravitreal injection of taxol reduces the incidence and extent of proliferative vitreoretinopathy in an animal model. It has been administered intravenously to patients with advanced ovarian cancer and metastatic breast cancer. There has been concern that supplies of taxol might be threatened because of the need to kill the endangered Western yew tree to obtain taxol. Recent advances in synthesizing semisynthetic taxol from the leaves of the yew tree, the production of taxol by plant cells in culture, and near success at de novo synthesis make future prospects for obtaining the drug excellent. Its potency, unique mechanism of action, and prolonged effect all make taxol an attractive candidate for further experimental study and possible clinical use.

**Key Words**
filtration surgery, taxol, etoposide, polyanhydride, monkeys

**References**


