An Intravitreal Sustained-Release Triamcinolone and 5-Fluorouracil Codrug in the Treatment of Experimental Proliferative Vitreoretinopathy

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Objective: To determine the efficacy and pharmacokinetics of an intravitreal sustained-release triamcinolone acetonide and 5-fluorouracil (TA/5-FU) codrug in the treatment of experimental proliferative vitreoretinopathy (PVR).

Methods: The therapeutic efficacy of the TA/5-FU codrug was determined in a rabbit model that simulates human PVR. Intravitreal levels of triamcinolone and 5-fluorouracil were measured at different time points and drug release in vitro was tested. Toxic effects were evaluated by electroretinography, clinical examination, and light microscopy.

Results: Both the severity of PVR grade and the percentage of eyes with moderate or worse tractional detachment were significantly less in eyes treated with the codrug. The therapeutic effect of the intravitreal codrug was paralleled by sustained intravitreal levels of triamcinolone and 5-fluorouracil. There were no drug-related toxic effects evident on clinical or histopathologic examination of eyes containing the TA/5-FU codrug.

Conclusions: The intravitreal sustained-release TA/5-FU codrug effectively inhibits the progression of PVR in a rabbit model that closely resembles PVR in humans. The TA/5-FU codrug may simultaneously target different components of the wound-healing response.


Proliferative vitreoretinopathy (PVR) refers to the migration and proliferation of cells into the subretinal space, vitreous cavity, and onto the retinal surface and undersurface. Subsequent collagen production and cell-mediated contraction of the collagenous scar leads to retinal detachment and loss of vision. Although refinements in surgical techniques and equipment have improved the success rate of surgery to repair retinal detachment in recent years, recurrence due to re-proliferation is not uncommon and remains the leading cause of failure of retinal reattachment surgery. A major challenge in the successful long-term therapy of PVR is stabilization of anatomical reattachment through pharmacological manipulation of the wound-healing response. A variety of pharmacological adjuncts have been evaluated to reduce the proliferation of fibrous tissue within the eye both in animal models and in limited human trials. When delivered by intravitreal injection, the short half-life of these agents necessitates frequent injections, thereby limiting their clinical utility. When administered systemically, it is difficult to achieve adequate intraocular drug levels without producing systemic toxic effects. Proliferative vitreoretinopathy is limited to the eye, and re-proliferation and recurrent retinal detachment occur within the first few months after the initial retinal reattachment surgery; thus, an intraocular drug delivery system that maintained therapeutic levels in the eye for several months would obviate the need for repeated intravitreal injections and would avoid toxic effects associated with systemic administration.

Both triamcinolone acetonide and 5-fluorouracil individually inhibit PVR in a rabbit model. We hypothesized that a device that contained both triamcinolone and 5-fluorouracil in combination may be more effective than treatment with either agent alone. In preliminary studies, we demonstrated the efficacy of a corticosteroid and 5-fluorouracil conjugate coated with polyvinyl alcohol polymer in the treatment of experimental PVR. The codrug represents a novel drug delivery system that is synthesized by linking a triamcinolone acetonide moiety to 5-fluorouracil via a modified labile carbonate bond. Once in the vitreous, dissolution of the conjugate followed by rapid hydrolysis allows both active components to be released in an...
MATERIALS AND METHODS

All animal experiments were conducted in accordance with the guidelines set forth by the Association for Research in Vision and Ophthalmology, Rockville Pike, Md, for the use of animals in ophthalmic and vision research. Experimental manipulations were performed on right eyes only. New Zealand white rabbits of either sex weighing approximately 1.5 to 2 kg were used for this study.

Experiments were divided into 3 parts as follows. Part 1 (41 rabbits): the therapeutic efficacy of 2.5 mg (group 1) or 10 mg (group 2) of TA/5-FU codrug powder injected intravitreally was compared with controls in the treatment of experimental PVR.

Part 2 (30 rabbits): the therapeutic efficacy of one 2.5-mg TA/5-FU codrug pellet (group 3) or three 3.3-mg pellets (group 4) implanted intravitreally was compared with controls in the treatment of experimental PVR.

Part 3 (15 rabbits): in vivo pharmacokinetics and toxic effects of the 10-mg TA/5-FU codrug suspension in normal rabbit eyes were determined. The in vitro release rates of the codrug powder and pellet were also measured.

PREPARATION OF TA/5-FU POWDER FOR INJECTION

A preweighed quantity of codrug powder was placed in a Teflon injection catheter, then connected to a syringe that contained 0.85 mL of hyaluronic acid (Provisc, Alcon, Fort Worth, Tex). The hyaluronic acid was used to mechanically push codrug powder through the injection catheter into the vitreous cavity through a sclerotomy.

PREPARATION OF TA/5-FU PELLETS FOR IMPLANTATION

Implantable sustained-release pellets containing 2.5 mg or 3.3 mg of the TA/5-FU codrug were prepared by direct compression of the powdered codrug into a 1.5-mm disk with a customized press (Parr Instruments, Moline, Ill).

PVR INDUCTION

Seventy-one New Zealand white rabbits underwent pars plana lensectomy, core vitrectomy, and penetrating retinal endodiathermy to induce PVR as previously described. Briefly, animals were anesthetized with an intramuscular injection of 0.3 mL of ketamine hydrochloride (100 mg/mL; Fort Dodge Laboratories, Fort Dodge, Iowa) and 0.1 mL of xylazine hydrochloride (100 mg/mL; Miles Inc, Shawnee Mission, Kan) per kilogram of body weight. A 5-mm peritomy was made at the superotemporal and superonasal quadrant of the right eye.

Sclerotomies were created after application of the endodiathermy probe to the retina for approximately 1 second. A sclerotomy was enlarged to a length of 2.5 mm with a keratome. Rabbits in treatment groups underwent codrug powder injection or pellet implantation through the enlarged sclerotomy. Control rabbits received hyaluronic acid injection or sham operation alone.

DRUG INJECTION OR IMPLANTATION

In part 1, the codrug powder, prepared as described earlier, was injected into the midvitreous cavity of the right eye after endodiathermy under direct view with an operating microscope. To avoid codrug reflux after injection caused by increased intraocular pressure, the infusion light pipe was removed from the sclerotomy to allow spontaneous egress of fluid during the injection. Control rabbits received an injection of hyaluronic acid alone. In group 1, the 2.5-mg TA/5-FU codrug powder was compared with controls. Twenty-two rabbits were used for this portion of the study, including 11 treated and 10 control rabbits. In group 2, 10-mg TA/5-FU codrug powder was compared with controls. Nineteen rabbits were used for this portion of the study, including 9 treated rabbits and 10 control rabbits.

In part 2, the codrug pellet, prepared as described earlier, was implanted into the vitreous base of the eyes of the experimental rabbits after endodiathermy. The implanted pellet remained in the vitreous base, and did not migrate posteriorly. Control rabbits underwent a sham operation alone. In group 3, implantation of one 2.5-mg pellet of TA/5-FU codrug was compared with controls. In group 4, implantation of three 3.3-mg pellets of TA/5-FU codrug was compared with controls. Thirty rabbits were used for this portion of the study, including 11 that received 1 pellet, 10 that received 3 pellets, and 9 controls. The sclerotomies were closed with 7-0 polyglactin 910 sutures. One drop (≈20 µL) of 0.3% gentamicin solution was instilled into the eye after surgery for infection prophylaxis. To maintain pupil dilation, 1 drop of 1% atropine solution was instilled into the eye after surgery for infection prophylaxis.

RESULTS

CLINICAL OBSERVATIONS

In part 1 (TA/5-FU codrug powder), the severity of PVR was significantly less in both experimental groups as compared with controls from week 4 to week 12 (P < .01 in group 1, P < .05 in group 2, Mann-Whitney U test) (Figure 2, A and B). In group 1 (2.5 mg of codrug powder), the percentage...
instilled daily for 1 week, then every other day for a second week.

PVR GRADING AND CLINICAL EXAMINATION

Pupils were dilated with 1 drop each of 2.5% phenylephrine and 1% tropicamide. The PVR grade was scored in a masked fashion weekly for 12 weeks by 2 independent observers using a classification system previously described by Hida and associates (Table). The PVR grade for each hemiretina was determined, and the 2 grades were then summed to obtain a total eye score (Figure 1). On week 12, all animals were killed under anesthesia using an intracardiac injection of pentobarbital sodium (Anpro Pharmaceuticals, Arcadia, Calif).

IN VIVO PHARMACOKINETICS

The right eye only of 12 New Zealand white rabbits (weight, 1.5-2.0 kg) was used for pharmacokinetic analysis. Anesthesia was performed with an intramuscular injection of ketamine and xylazine as described earlier. A 1.5-mm sclerotomy was created 1.0 mm posterior to the limbus in the superior temporal quadrant. To soften the eye, a 26-gauge needle was inserted into the anterior chamber at the limbus and 0.1 mL of aqueous removed. Ten milligrams of the TA/5-FU codrug was suspended in 0.1 mL of hyaluronic acid (Healon, Pharmacia Inc, Columbus, Ohio) and injected into the midvitreous through a 19-gauge needle inserted through the sclerotomy. The tip of the needle was directly viewed with an operating microscope during injection of the codrug. The sclerotomy was closed with 7-0 polyglactin 910 sutures. After injection, the codrug was initially suspended in the midvitreous. Although it remained in this location in a few eyes, the codrug settled into the inferior vitreous cavity in most eyes.

Animals were killed at 1, 7, 42, and 84 days. Three animals were killed at each time point by intracardiac injection of pentobarbital. The eyes were enucleated and immediately frozen at −80°C. The tissues were then prepared for assay as described below.

DRUG LEVEL ANALYSIS

The vitreous was dissected from the frozen globe. Triamcinolone, 5-fluorouracil, and intact codrug levels in 100 μL of vitreous were determined. Assays were performed using a fully automated high-pressure liquid chromatography system (Hitachi Scientific Instruments, Mountain View, Calif) with a C-18 reverse-phase column with a C-18 guard column. Acetonitrile and 0.02% sodium acetate (pH 4.0) were used in the mobile phases. Detection was by UV light at 266 nm for 5-fluorouracil and 234 nm for triamcinolone and intact codrug.

ASSessment of Toxic Effects

Toxic effects of the TA/5-FU codrug were determined on eyes used for the pharmacokinetic analysis (described above). Slit-lamp examination and indirect ophthalmoscopy were performed immediately prior to death, ie, at 1, 7, 42, and 84 days.

Scotopic electroretinography (ERG) was performed in both eyes of 3 additional rabbits prior to injection of 10 mg of codrug powder in the right eye. Postinjection ERG was performed at 14, 28, and 63 days. Electroretinography examinations were recorded with the use of contact lens electrodes (ERG-Jet, Universal SA, La Chaux-de-Fonds, Switzerland) with a 2-channel clinical signal averager (No. 5200, Cadwell, Kennewick, Wash) and a Ganzfeld flash unit (VPA-10, Cadwell). Scotopic ERGs, obtained after at least 30 minutes of dark adaptation, were elicited at 0.34 Hz. For each ERG, 20 stimulus presentations were averaged. To minimize the effect of individual and daily variation on the ERG, the ratio of the B-wave amplitude of the experimental (right) eye to the B-wave amplitude of the control (left) eye was determined. When the amplitude of the experimental and control eyes are equal, the ratio equals 1. A decrease in the ratio reflects a relative decrease in the B-wave amplitude of the experimental eye.

The rabbits used for ERG analysis were killed at 65 days for histopathologic analysis. The codrug-injected eyes were immediately enucleated and a 3-clock-hour circumferential incision was created 1.0 mm posterior to the limbus. The eyes were fixed by immersion in 2% glutaraldehyde in 0.1-mol/L sodium cacodylate buffer (pH, 7.4) and processed in paraffin. Sections were stained with hematoxylin-eosin and examined by light microscopy.

IN VITRO PHARMACOKINETIC STUDY

Drug release from the 2 formulations of codrug (2, 5, and 10 mg of codrug powder; 1, 4, and 8 mg of codrug pellet) were determined by placing the codrug into a microcentrifuge tube containing 1.0 mL of 0.1-mol/L phosphate buffer solution (pH, 7.4; 37°C). Every 24 hours the tubes were centrifuged and 0.5 mL of the supernatant was removed for analysis by reverse-phase high-pressure liquid chromatography as described earlier. Then, 0.3 mL of fresh buffer was added and the determination continued for 32 days.

STATISTICAL ANALYSIS

A Mann-Whitney U nonparametric test was used to compare the difference in median clinical grade of PVR between experimental and control groups. The chi-square test was used to compare the difference in the number of eyes with retinal detachment between experimental and control animals. A paired 2-tailed t test was used to compare the ERG B-wave amplitude ratio before and after codrug injection.

**Clinical Grading of Proliferative Vitreoretinopathy**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal retina</td>
</tr>
<tr>
<td>1</td>
<td>Contraction of medullary ray</td>
</tr>
<tr>
<td>2</td>
<td>Mild tractional elevation of retina (&lt; 1 DD)*</td>
</tr>
<tr>
<td>3</td>
<td>Moderate tractional elevation (1-2 DD)</td>
</tr>
<tr>
<td>4</td>
<td>Severe tractional elevation (≥ 2 DD)</td>
</tr>
<tr>
<td>5</td>
<td>Bullous retinal detachment</td>
</tr>
</tbody>
</table>

*DD indicates disc diameter.
tractional detachment or worse were significantly less in group 4 (three 3.3-mg pellets) than in controls from week 5 to week 12 (P<.01) (Figure 2, D and Figure 3, D). In group 3 (one 2.5-mg pellet), the severity of PVR was less in treated eyes than in controls at all time points, but the difference was not statistically significant (P=.07 at week 11 and 12, Mann-Whitney U test) (Figure 2, C). Nevertheless, the percentage of eyes with moderate tractional detachment or worse was statistically significantly less in treated eyes (40%) than in controls (90%) at week 8 and thereafter (P<.05, χ² test) (Figure 3, C).
In eyes receiving the 10-mg codrug suspension, the vitreous concentration of free TA remained above 200 µg/mL for the first 6 weeks of the study, and then declined to 36 µg/mL by the 12th week. 5-Fluorouracil levels were above 50 µg for the first 6 weeks and remained above 10 mg/mL thereafter (Figure 4). The amount of intact codrug in the vitreous cavity gradually declined from 10 µg to 2.9 mg by week 6 and was still measurable (0.47 mg) by week 12. Ophthalmoscopy was used to determine whether codrug powder was visible clinically. At week 12, a moderate amount of codrug powder was visible in the vitreous cavity of 2 eyes and a trace amount was visible in 1 eye.

**IN VITRO PHARMACOKINETICS**

In vitro codrug powder (2, 5, and 10 mg) provided sustained release of 5-fluorouracil for up to 33 days (Figure 5, A). The surface areas of the 1-, 4-, and 8-mg codrug pellet are 9.0, 21.8, and 43.5 mm², respectively. The 5-fluorouracil release from codrug pellets (1-, 4-, and 8-mg) was proportional to their surface area and constant over this period (Figure 5, B and C).

**CODRUG TOXICITY**

There was no evidence of toxic effects based on the clinical examinations. Specifically, no inflammation, cataract formation, or retinal abnormalities developed.
Electroretinograms showed no evidence of codrug toxic effects. The B-wave amplitudes remained normal throughout the study. Ratios of the ERG B-wave amplitudes of the injected eye to the B wave of the contralateral (control) eye were not statistically different when pre-injection values were compared with postinjection values ($P = .27$ at day 63, paired $t$ test) (Figure 6).

On histopathologic analysis, the ciliary body and retina were normal by light microscopic examination (Figure 7). No sign of retinal necrosis, photoreceptor cell loss, cystic degeneration, inflammatory cell infiltration, or hypocellularity of nuclear layers was observed.

**COMMENT**

The results of our study demonstrate that injectable or implantable TA/5-FU codrug effectively inhibits the progression of PVR in a rabbit model. Both the severity of PVR grade and percentage of eyes with moderate tractional detachment or worse were significantly less in experimental eyes than control eyes in all groups. There was no evidence of drug-related toxic effects by ERG or on clinical or histopathologic examination of eyes containing the TA/5-FU codrug.

Ideally, sustained drug-delivery systems should release the drug over a period that corresponds to the duration of disease activity. The earliest signs of PVR, cells in the vitreous cavity and subretinal space, are visible in most patients soon after rhegmatogenous retinal detachment. Progression to formation of fibrocellular membranes and subsequent cell-mediated contraction of these membranes occurs over the ensuing 6 to 12 weeks after surgery to repair the retinal detachment. The codrug systems described in the current report produced a therapeutic effect for the duration of the 12-week observation period. This effect was paralleled by sustained intravitreal levels of triamcinolone and 5-fluorouracil. During the first 6 weeks the vitreous concentration of free triamcinolone remained above the concentration of drug required to produce 50% inhibition of fibroblast cell growth (the 50% inhibition level for triamcinolone is 150 µg/mL). The concentration of free 5-fluorouracil remained above the 50% inhibition level for fibroblast cell growth (0.3 µg/mL) for the entire 12-week duration of the experiment. Similarly, the in vitro pharmacokinetic analysis showed that the TA/5-FU codrug suspension produced sustained drug release over an extended period. Together, these data suggest that the corticosteroid–5-fluorouracil codrug pharmacokinetic profile would be advantageous in the treatment of PVR, a disease that typically runs its course over a 6- to 12-week period.

The injectable codrug powder had a different pharmacokinetic profile than the compressed codrug pellet. As demonstrated by the in vitro studies, drug release rate is directly proportional to the surface area. It is possible to achieve a much higher surface area with the codrug powder than with the compressed pellet. Thus, for an equivalent quantity of codrug over a given period, the amount released from the codrug powder is higher than that released from the pellet. Furthermore, the codrug powder followed logarithmic rather than linear release kinetics; this produced high initial drug levels in the vitreous, which subsequently logarithmically declined. In contrast, the codrug pellet released the drug in a linear manner. We hypothesize that these pharmacokinetic profiles accounted for the observation that the 2.5 mg of codrug powder more effectively inhibited PVR in the rabbit eye than the 2.5-mg codrug pellet. It is likely that the initial pulse of triamcinolone and 5-fluorouracil provided by the powdered codrug rapidly suppressed cellular proliferation and inflammation, and that lower drug levels were necessary to maintain suppression until cellular proliferation ceased.
In contrast, the constant drug levels obtained with the 2.5-mg codrug pellet may have been insufficient to completely suppress cellular proliferation and inflammation at a time when disease activity was maximal.

The powdered codrug has several potential advantages over the compressed pellet and polymeric delivery devices. As discussed earlier, the kinetic release profile of the powdered codrug may be advantageous in the treatment of PVR. In addition, because the powdered codrug is given as an injection, it is possible to administer the drug without creating a large surgical wound. In contrast, the codrug pellet described in the current report and polymeric devices described previously must be inserted through a relatively large surgical wound. Despite these advantages, if the powdered codrug were used to treat PVR, drug dispersion into the vitreous cavity could temporarily diminish the physician’s view of the patient’s retina during postsurgical follow-up examinations. In contrast, the codrug pellet provides local drug delivery without obscuring the retinal view. Both formulations are improvements over the polymeric device described in our previous report; the compressed pellet and codrug powder serve as their own delivery devices and no residual foreign body remains in the eye after the codrug has disappeared. In contrast, the nonerodible device described in our previous reports remains within the eye permanently.

The technique used to deliver the injectable form of the codrug is an improvement over that described in our previous report. In the previous experiments, the codrug powder was suspended in balanced isotonic saline solution or hyaluronic acid prior to injection. After

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Figure 6. Representative scotopic electroretinography in eye receiving 10 mg of triamcinolone acetonide and 5-fluorouracil codrug. The ratios of the electroretinographic B-wave amplitude of the right eye (injected) to the left eye (control) were not statistically different when preinjection values (left) were compared with postinjection values (right) at day 63 (P=.27).

Figure 7. Histopathologic appearance of the retina at medullary ray (left) and ciliary body (right) at week 9 after injection of 10 mg of triamcinolone acetonide and 5-fluorouracil codrug (hematoxylin-eosin, ×250).
injection of this suspension, there was frequently residual codrug adherent to the walls of the syringe. Thus, the quantity of injected codrug was somewhat variable. In the current study, hyaluronic acid was used to mechanically push the desired quantity of codrug powder out of the syringe into the vitreous cavity. With this technique, there was no residual codrug in the syringe. Thus the quantity of the codrug injected was reproducible and accurate.

In previous investigations, we chose the fibroblast injection model of PVR to test the efficacy of a corticosteroid–5-fluorouracil conjugate.22,23 However, this model has several limitations. For example, PVR develops more rapidly in the fibroblast-injection model than in humans.24,25 Unlike human PVR, in the fibroblast-injection model PVR is not initiated by a retinal tear and neovascularization is a prominent component. Furthermore, drug therapy may inhibit injected fibroblasts, which would otherwise induce proliferation of endogenous cells. Finally, because of the severe nature of PVR in this model, lack of drug efficacy does not necessarily mean that the drug would be ineffective in humans. For these reasons, in this investigation we chose a refined model of PVR that has several advantages over that used previously. In the refined model, first described by Iverson and associates,19 PVR is produced by creating full-thickness retinal defects accompanied by breakdown of the blood-retinal barrier induced by lensectomy, vitrectomy, and penetrating diathermy. In this model, proliferation is induced only by endogenous cells, neovascularization is not a prominent component, and PVR occurs reproducibly in more than 90% of animals. Most importantly, unlike the rapid time course of PVR observed in the fibroblast-injection model, with the refined model the time course for development of PVR (4-12 weeks) is very similar to that observed in humans. This time course allowed us to test codrug efficacy over a protracted period, which was not possible with the fibroblast-injection model.

The TA/5-FU codrug conjugate was not toxic to the normal rabbit eye. We chose to evaluate the safety of the codrug in normal animal eyes rather than eyes with retinal detachment and PVR. It is recognized that the potential toxic effects may differ in normal eyes and in eyes with a retinal detachment and PVR. However, eyes with retinal detachment often develop decreased intraocular pressure, loss of ERG B wave, retinal edema, and loss of photoreceptor outer segments—changes that can mimic the toxic effects from an intraocular drug. Therefore, because it may be virtually impossible to distinguish drug effects from those induced by the retinal detachment, experiments conducted in normal eyes have generally been considered an appropriate means to evaluate toxic effects.26

Clinically, one could envision several possible means of delivering the TA/5-FU codrug for prophylaxis or treatment of PVR. Evaluation of toxic effects in the primate eye would be necessary before beginning human clinical trials. Assuming a lack of toxic effects, one could consider injecting the codrug prophylactically into the vitreous cavity at the time of primary scleral buckle to repair a rhegmatogenous retinal detachment. Alternatively, the codrug might be placed in the eye at the conclusion of vitrectomy to repair a primary or recurrent rhegmatogenous retinal detachment in the presence or absence of PVR. In this situation, gas is often used as a vitreous substitute to provide an extended retinal tamponade. The pharmacokinetics of the codrug in an eye containing gas are unknown. Although they are beyond the scope of the current report, experiments to determine the toxic effects of the codrug in primates and the pharmacokinetics of the codrug in a gas-filled eye are under way in our laboratory.

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REFERENCES


Error in Figures. Due to errors in cropping of figures during processing for publication in the article titled “A Comparison of Retinal Morphology Viewed by Optical Coherence Tomography and by Light Microscopy,” in the November issue of the Archives (Arch Ophthalmol. 1997;115:1425-1428), the upper portion of the top image in Figure 1 is missing and the left and right parts of Figure 2 are incorrectly matched. Figures 1 and 2 are reprinted correctly here. The Journal regrets the errors.