Intravitreal Sustained Release Corticosteroid-5-Fluoruracil Conjugate in the Treatment of Experimental Proliferative Vitreoretinopathy

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Purpose. Proliferative vitreoretinopathy (PVR) remains the most common cause of failed retinal detachment (RD) surgery. The authors compared the effectiveness of two intraocular sustained-release codrugs in suppressing PVR in a rabbit model: a surgically implantable pellet releasing 5-fluorouracil (FU) and dexamethasone (DX) for 1 week and an injectable intravitreal sustained-release suspension releasing 5-FU and triamcinolone acetonide for 1 month.

Methods. Sustained-release devices and suspensions were prepared to deliver equimolar quantities of corticosteroid and 5-FU. In group 1, devices were implanted surgically into the vitreous of the right eye of 10 New Zealand White rabbits. Ten control rabbits received surgical implantation of the suture only. In group 2, drug suspension was injected into the vitreous of the right eye of 10 New Zealand White rabbits. Ten control rabbits received injection of the vehicle only. One day later, each rabbit was injected intravitreally with 250,000 homologous rabbit dermal fibroblasts. Severity of PVR was graded clinically by two masked observers on days 3, 7, 10, 14, 21, and 28.

Results. In group 1, clinical severity of PVR was less in the experimental group than in the control group at all time points; this was only statistically significant on day 10 (P = 0.04). Six eyes developed moderate to severe tractional RD or bullous RD in the control group by day 10 compared with none in the experimental group (P = 0.01). In group 2, the median clinical grading of eyes in the experimental group was significantly less than that in the control group at all time points through day 21 (P < 0.01).

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Because PVR is limited to the eye and because reproliferation and recurrent retinal detachment occur in the first few months after the initial retinal reattachment surgery, an intraocular device providing sustained delivery of drugs may be an effective means of eliminating systemic toxicity while providing therapeutic intraocular drug levels. Recently, an implantable, nonerodible intraocular device has shown promise in the treatment of experimental uveitis, and a similar device has been shown to be effective in the treatment of cytomegalovirus retinitis in humans. Although these devices are effective, they are not bioerodible; this may limit their clinical application to chronic diseases requiring long-term (months or years) delivery. In PVR, chronic delivery may not be required, and a short-term, bioerodible device may be more appropriate. This study was designed to evaluate the efficacy of two codrugs in preventing the development of PVR in a rabbit model. The codrugs represent novel drug delivery systems composed of an antimetabolite (5-fluorouracil [FU]) covalently linked to a corticosteroid (dexamethasone [DX] or triamcinolone acetonide [TA]). When implanted in the eye, these systems slowly dissolve and hydrolyze, giving sustained release of their parent compounds.

METHODS

All animal experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental manipulations were performed on right eyes only. Forty New Zealand white rabbits of either sex weighing approximately 2 kg were used for this study.

Synthesis of Codrugs

The 5-FU corticosteroid codrugs were synthesized as follows. Briefly, 5-FU was reacted with formaldehyde at 60°C to generate bis and mono (hydroxymethyl)-5-fluorouracil. In a separate reaction, DX or TA was reacted with phosgene to generate the 21-chloroformate. Reacting the chloroformate with bis or mono (hydroxymethyl)-5-fluorouracil generates the codrug (Figs. 1, 2).

Preparation of DX-5-FU Codrug Pellet

Implantable sustained-release devices containing 2.5 mg of the DX-5-FU conjugate were prepared by direct compression of the powdered codrug in a 1.5 mm customized Parr Instruments (Moline, IL) press. Each pellet contained the equivalent of 1.9 mg of DX and 0.6 mg of 5 FU. Pellets were then secured to a 7-0 nylon suture using a trace amount of ophthalmic-grade silicone (supplied by Chiron Vision, Irvine, CA).

Preparation of TA-5-FU Codrug Suspension

As an alternative to the DX-5FU device, an injectable system was prepared using the TA-5-FU codrug. TA is significantly less soluble than DX, so the TA-5-FU codrug could be expected to be less soluble and, therefore, longer lasting, than the DX-5-FU codrug. Pow
dered TA-5-FU (2.5 mg) was suspended in balanced salt solution to a concentration of 2.5 mg in 0.1 ml.

**Determination of In Vitro Drug Release**

Drug release from the two formulations was determined by either injecting or immersing the drug into 1 ml of 0.1 M phosphate buffer (pH 7.4, 37°C) in microcentrifuge tubes. 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 (HPLC). Then, 0.5 ml of fresh buffer was added, and the determination was continued. Assays were performed using a fully automated Hitachi (San Jose, CA) HPLC system with a C-18 reverse-phase column with a C-18 guard column. Acetonitrile and 0.02% sodium acetate (pH 4) were used in the mobile phases. Detection was by ultraviolet light at 266 nM for 5-FU and 234 nM for DX, TA, and intact codrug.

**Drug Implantation**

In group 1, intravitreal sustained-release DX-5-FU devices were implanted surgically into the right eyes of 10 New Zealand White rabbits (treated group) according to a previously described method. Briefly, animals were anesthetized with an intramuscular injection of 0.3 ml ketamine hydrochloride (100 mg/ml) and 0.1 ml xylazine hydrochloride (100 mg/ml) per kilogram. A 5 mm peritomy was made at the superotemporal quadrant of the right eye. A 3-mm sclerotomy was created 2 to 3 mm posterior to the limbus. The device was inserted into the vitreous cavity through the sclerotomy and suspended at the sclerotomy site by its own 7-0 nylon suture. The sclerotomy wound and the peritomy were then closed with 7-0 vicryl sutures. The other 10 rabbits underwent surgical implantation of the silicone vehicle alone and served as a control group. Although previously published reports of studies using a larger, nonerodible device have used prophylactic cryotherapy to the superior temporal quadrant of the right eye to prevent retinal detachment associated with the sclerotomy, we did not find this procedure necessary because of the smaller sclerotomy needed for the codrug devices.

In group 2, a 5-mm peritomy was made at the superotemporal quadrant of the right eye. A 3-mm sclerotomy was created 2 to 3 mm posterior to the limbus. A suspension consisting of 2 mg of a TA-5-FU conjugate in 0.1 ml balanced salt solution was injected with a 19-gauge needle through the sclerotomy into the vitreous of the right eye of 10 rabbits. Ten rabbits received injection of the balanced salt solution alone and served as a control group. The sclerotomy and the peritomy were then closed with 7-0 vicryl sutures. In all animals, one drop of topical 0.3% gentamicin solution was instilled into the eye postoperatively for infection prophylaxis.

**TABLE 1. Clinical Grading of Proliferative Vitreoretinopathy**

<table>
<thead>
<tr>
<th>Stage</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 (&gt;2 DD)</td>
</tr>
<tr>
<td>5</td>
<td>Bullous retinal detachment</td>
</tr>
</tbody>
</table>

**Proliferative Vitreoretinopathy Induction**

Experimental PVR was induced according to a previously published protocol. Briefly, tissue-cultured homologous rabbit dermal fibroblasts were grown to confluence. Cells were trypsinized and suspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 0.22 mg/ml gentamicin sulfate, 89 U/ml penicillin sodium, 89 μg/ml streptomycin, and 0.22 μg/ml amphotericin B. The suspended cells were counted and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed, and the cells were resuspended in enough phosphate-buffered saline to achieve a final cell density of 250,000 per 0.1 ml.

The rabbits were anesthetized the day after drug implantation, and 250,000 fibroblasts prepared as above were injected directly over the optic disc. This injection was given using a 30-gauge needle that was passed adjacent to the implant site. Before injection of the cells, the needle tip was swept over the disc several times to disrupt the vitreous. During this procedure, the surgeon had no knowledge of whether the rabbit had received active drug or vehicle alone.

**Clinical Examination**

Pupils were dilated with one drop each of phenylephrine hydrochloride (2.5%) and tropicamide (1%). In group 1, there was an observable difference between the DX-5-FU devices and the control devices, which contained no drug core. To minimize bias, the status of the inferior retina, disc region, and nasal ray were determined before evaluation of the temporal ray and superior temporal quadrant. The grade of PVR for each hemiretina was scored, and the two scores were totaled to obtain the median clinical grade.

**Statistical Analysis**

The difference in median clinical grade was computed using a Mann–Whitney test for nonparametric data. The difference in percentage of detachment was com-
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Dexamethasone/5-FU Device

Experimental
Control

FIGURE 3. (A) Median clinical grade of eyes in group 1 (dexamethasone–5-fluorouracil device). (B) Percentage of eyes in group 1 with moderate traction retinal detachment or worse.

RESULTS

For group 1 (DX-5-FU codrug pellet), the median clinical grade of eyes in the control group was higher than that of the experimental group at all time points (Fig. 3A). This difference was statistically significant only on day 10 ($P = 0.04$, Mann–Whitney test). The percentage of eyes with moderate tractional detachment (grade 3 in at least one ray) or worse was greater in the control group than in the experimental group at all time points (Fig. 3B). Again, this difference was only statistically significant on day 10 ($P = 0.01$, chi-square analysis).

It was observed clinically that the DX-5-FU device fully released in approximately 1 week (as indicated by visualization of the drug). In vitro release studies showed that the higher solubility DX-5-FU codrug pellet dissolved over approximately 1 week in buffer, releasing both DX and 5-FU (Fig. 4). Intact codrug could not be detected in the supernatant at any stage, suggesting rapid hydrolysis of the codrug once in solution. We concluded that the rapid depletion of the drug supply allowed the disease to progress in the later time points because of insufficient residual quantities of drug.

Therefore, the drug was reformulated as a lower solubility TA-5-FU codrug suspension. In vitro analysis revealed that this system, despite the increased surface area, dissolved more slowly and gave longer sustained release than DX-5-FU (1 month versus 1 week). Group 2 animals received the TA-5-FU codrug suspension. In this group, results were more favorable. Once again, the median clinical grading of eyes in the control group was higher than in the experimental group at all time points (Fig. 5A). This difference was statistically significant at all time points through day 21 ($P \leq 0.01$, Mann–Whitney test). Similarly, the percentage of eyes with moderate tractional detachment (grade 3 in at least one ray) or worse was greater in the control group than in the experimental group at all time points (Fig. 5B). This difference was statistically significant on days 6 and 14 ($P < 0.05$, chi-square analysis).

DISCUSSION

The idea of using 5-FU in combination with a corticosteroid was suggested shortly after both agents were found to be effective in treating experimental PVR. To take advantage of the best characteristics of these two drugs while minimizing their individual liabilities, we combined corticosteroid and 5-FU as a conjugate. The use of a conjugate drug is a novel approach that allows sustained release of both drugs. The drugs become relatively insoluble when covalently linked

FIGURE 4. Release of 5-fluorouracil (FU) from dexamethasone–5-FU pellets versus triamcinolone–5-FU suspension in vitro. Note the rapid release from the pellet.
through the carbonate bond. Hydrolysis of this bond allows corticosteroid and 5-FU to be released slowly into the vitreous in equimolar quantities. The DX-5-FU conjugate pellet released 5-FU and DX over 1 week in vitro. When administered in a rabbit model of PVR, the conjugate performed well, reducing the incidence of moderate retinal detachment from 70% to 20% on day 13. However, by day 20, the retinal detachment rate in the experimental group was up to 40%. The TA-5-FU conjugate gave considerably longer sustained release in vitro than the more soluble DX-5-FU conjugate, and the results obtained were more favorable. The TA-5-FU suspension reduced the incidence of moderate to severe retinal detachment from 89% to 30% on day 14. At no time through day 28 in the suspension group was the incidence of retinal detachment higher than on day 14. This trend was associated with a regression of PVR severity in the experimental and the control groups at the later time points.

Both dexamethasone alcohol and triamcinolone acetonide have been shown to reduce the incidence of retinal detachment in a rabbit model of PVR.14,15 Although corticosteroids have some limited antiproliferative properties,16 they may play a more important role by preventing the access of inflammatory mediators by stabilization of the blood–retinal barrier.17,18 T-lymphocytes have been identified in excised membranes from cases of PVR.19,20 In addition, elevated levels of certain cytokines, such as tumor necrosis factor α21 and interleukin-1,22 have been found in the vitreous of patients with PVR. Glucocorticoids inhibit TNF-α and IL-1 expression,23,24 and can inhibit T-cell proliferation.25

Despite the advantages of corticosteroids in suppressing the inflammatory arm of the wound-healing response, they are potentially limited in their clinical effectiveness because of their weak antiproliferative properties. The corticosteroid–5-FU codrug retains the anti-inflammatory properties of corticosteroids while adding the powerful antiproliferative properties of the antimetabolite.

Ophir13 and Blumenkranz et al26 demonstrated that a single 1 mg intravitreal injection of the synthetic pyrimidine analog 5-FU could reduce the rate of tractional retinal detachment in a rabbit model of PVR created by injection of heterologous fibroblasts. 5-FU is a potent inhibitor of rabbit dermal fibroblast proliferation in cell culture and is well tolerated in the rabbit when given intravitreally in dosages as high as 1 mg.27 The theoretical advantages of 5-FU over the synthetic glucocorticosteroids include a greater reduction of rabbit fibroblast proliferation in cell culture than corticosteroids on a unit-weight basis and no paradoxical stimulatory effect on fibroblastic proliferation in cell culture at low concentrations.

Nevertheless, 5-FU has limited clinical usefulness because of its pharmacokinetics. In the normal rabbit, the half-life of 5-FU after intravitreal injection is 7.7 hours, whereas in aphakic vitrectomized animals, the half-life is reduced to 3.2 hours. Therapeutic levels are present in phakic rabbit eyes for 72 hours after a single 1 mg intravitreal injection; in aphakic vitrectomized eyes, therapeutic levels are maintained for only 12 to 24 hours.28 If we extrapolate to the clinical scenario, a single intravitreal injection of 5-FU immediately after vitrectomy probably would not result in therapeutic drug levels when the proliferation is most likely to be occurring. Using cultured retinal pigment epithelial cells, Stern et al39 showed in an animal model after vitrectomy that it was necessary to give repeated injections of 0.5 mg 5-FU every 24 hours for 7 days to achieve a nontoxic, yet clinically significant, effect because of rapid clearance of the drug from the eye. Repeated injections are associated with the risk for endophthalmitis and retinal detachment, as well as with inconvenience and discomfort for the patient. Moreover, the drug is toxic to the cornea and retina when administered in higher dosages.30 Although the half-life of injected 5-FU is greatly extended by the intact vitreous and lens in the animal model,31 clini-
cally most PVR is seen in eyes that have already undergone vitrectomy. In addition, it is clear from recent animal studies that traction detachments may develop in eyes treated with 5-FU as late as 2 months after intravitreal fibroblast administration. These data suggest the possible need for a protracted treatment course.

Other investigators have explored various methods for delivering intraocular 5-FU over sustained periods of time, such as solid implants and microspheres, both of which use a biodegradable polymer as the delivery vehicle. Conjugates of 5-FU with either DX or TA have a distinct advantage over the use of either drug alone because they incorporate the properties of the parent compounds while allowing sustained delivery of both drugs over an extended period of time. Dexamethasone alcohol and triamcinolone acetonide are relatively lipophilic and, therefore, may be administered as a suspension, conjugated with 5-FU. The crystalline conjugate then acts as a depot, releasing both drugs into the vitreous over time. Thus, a sustained release is achieved without recourse to more traditional polymeric drug delivery systems, some of which have been shown to induce a mild localized foreign body reaction with glial proliferation and inflammatory reaction still visible histopathologically at 2 months. The advantage of the conjugate drug delivery system is that the implants or injections can be small (because no polymer is needed), and concerns of polymer biocompatibility are moot. Ideally, injection or surgical implantation of the drug could be performed at the end of the vitrectomy; the drug would then release slowly over the course of several weeks, obviating the need for supplemental therapy.

The current study is limited because the PVR model chosen, though traditional, is not ideal for simulating the human condition. We chose this model for ease of comparison with other animal studies in the literature. It is limited by the absence of vitrectomy and the absence of an extended intraocular tamponade, such as gas or silicone oil, which is typically used after surgery for PVR in humans. It is unclear how the release rates of the conjugate drugs would be impacted by these conditions. However, one advantage of the codrug system is that release of the parent compounds is determined by solubility. The concentration of the two drugs in the aqueous phase is constant, regardless of the gas fill; thus, there is theoretically less risk of generating toxic drug concentrations.

An additional limitation of this model is that the bolus injection of fibroblasts causes a rapidly developing PVR, unlike clinical PVR, which develops slowly over the course of 6 weeks. However, we speculate that the slow-release conjugate drug might be more effective in a PVR model that develops slowly over time than the fibroblast-injection model. Experiments using a more clinically relevant PVR model that takes full advantage of the codrug system are beyond the scope of this study but are under investigation.

The suspension codrug inhibited PVR slightly better than the implantable device. One possible reason could be that the PVR is more severe in the setting of an intraocular implant because the device serves as a scaffold for proliferation (Pearson PA, unpublished data, 1993). Another possible reason that the DX-5-FU conjugate did not inhibit PVR as effectively at the later time points is related to the pharmacokinetics of the drug. Co-drug release into solution occurs in two steps (Fig. 6). The first step, co-drug dissolution, is determined by the solubility of the compound and is the rate-determining step. The second step, co-drug hydrolysis, occurs rapidly. It was observed clinically that the DX-5-FU device fully released in approximately 1 week (as indicated by visualization of the drug). Our in vitro pharmacokinetic data suggested that the high solubility of the DX—5-FU pellet allowed the disease to progress in the later time points because of insufficient residual quantities of drug. The TA-5-FU suspension preparation has a lower solubility in vitro than the pellet and was designed to release at a controlled rate over a more extended period of time. Clinically, the TA-5-FU suspension was observed to release slowly over the duration of the study. This concurs with the in vitro evaluation of drug release from the two co-drug systems, the primary purpose of which was to provide data for optimizing the solubility profile of the drug. Eventually, a fuller evaluation of these compounds should be performed in vitrectomized eyes with an extended intraocular tamponade. These conditions are likely to have a large effect on vitreous
elimination rates and, hence, intravitreal drug levels. For this reason, a full in vivo pharmacokinetic study of the two dosage forms in this model was not performed. However, the current study indicates that prolonged release of 5-FU and triamcinolone (weeks) may be more effective than the release of dexamethasone and 5-FU over a shorter period of time (days). The suspension was effective in suppressing PVR for 21 days. We hope that future modifications of the drug will be even more effective over a longer time course.

In summary, both the intravitreal sustained release DX-5-FU device and the TA-5-FU suspension are effective in inhibiting the progression of PVR in a rabbit model. Combination therapy may target different components of the wound-healing process in this disease and is a promising approach to the pharmacologic treatment of PVR.

Key Words
antimetabolites, corticosteroids, drug delivery, proliferative vitreoretinopathy, retinal detachment

References


