Aclacinomycin A in the Treatment of Experimental Proliferative Vitreoretinopathy
Efficacy and Toxicity in the Rabbit Eye

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Purpose. Aclacinomycin A is an oligosaccharide anthracycline that, by contrast with daunomycin, lacks carcinogenicity. The authors evaluated the efficacy of aclacinomycin A in prevention of experimental proliferative vitreoretinopathy (PVR) and its toxicity on the rabbit retina.

Methods. Dutch-belted rabbit were used to create a model for traction retinal detachment. Seven to 10 days after vitreous gas compression, 25,000 homologous fibroblasts were injected into the vitreous cavity. Subsequently, the eyes received either sham injections or doses of 6, 30, or 60 nmol of aclacinomycin A, respectively. The fundus findings were documented on days 7, 14, and 28 after the fibroblast injection. The toxicity studies were conducted according to the same protocol as was used for the efficacy evaluation but without the fibroblast injection. Simultaneous electroretinograms were recorded on days 0, 3, 7, and 14 from the right eyes that were injected with 30 or 60 nmol of aclacinomycin A and the left eyes that were sham injected. Morphologic studies were conducted on the eyes enucleated on days 3, 7, and 14 after drug exposure.

Results. Intraocular administration of 30 nmol of aclacinomycin A on day 2 after fibroblast injection resulted in a detachment rate of 37.5% (controls, 100%; P < 0.01, by Fisher’s exact test). Administration of 60 nmol of aclacinomycin A 3 days after fibroblast injection resulted in a detachment rate of 26.7% (controls, 100%; P < 0.0001). Six nanomoles of aclacinomycin A 3 days after fibroblast injection had no effect. No electroretinogram changes were present in eyes treated with 30 nmol of aclacinomycin A. Such recordings from eyes exposed to 60 nmol of aclacinomycin A demonstrated decreased a- and b-waves on day 3; these completely recovered by day 7. Morphologic studies of these eyes revealed no damage to the retina.

Conclusions. These results suggest that aclacinomycin A should be considered an alternative to daunomycin for treatment of human PVR because, in addition to its lack of carcinogenicity, it shows good efficacy and causes less retinal toxicity. Invest Ophthalmol Vis Sci. 1993; 34:1753-1760.

Surgical treatment of proliferative vitreoretinopathy (PVR) has yielded increasingly higher success rates in recent years as a result of refinements in equipment and techniques and persistence with reoperations. However, certain PVR stages and clinical conditions are still difficult to treat, and recurrence of PVR is not rare despite good anatomic results immediately after surgery. The etiologic basis of PVR is intraocular cell proliferation and subsequent membrane formation.
and collagen deposition. Intraocular administration of antiproliferative drugs can prevent cell growth and thus decrease the incidence of postoperative PVR. Daunomycin is the only anthracycline that is currently applied in patients with an increased risk of PVR. Studies on retinal toxicity of daunomycin in rabbits revealed that even the smallest dose that was effective in preventing experimental PVR caused damage to the photoreceptors.

An additional obstacle to application of daunomycin in otherwise healthy patients is its high mutagenic and carcinogenic potential, as has been reported by numerous authors. N-alkylation of the aminosugar moiety of the anthracycline molecule results in a loss or a significant reduction of the mutagenicity and carcinogenicity. Aclacinomycin A is a dimethylated oligosaccharide anthracycline (Fig. 1) that has been shown to be noncarcinogenic in vitro and in animal models. Using a rabbit model, we determined effective doses of aclacinomycin A to prevent experimental PVR. The retinal toxicity was evaluated by electroretinography (ERG) and morphologic studies.

MATERIALS AND METHODS

All animal experiments were conducted in adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental manipulations were performed on right eyes only. Sixty-four Dutch-belted rabbits of both sexes weighing 1.5–2.0 kg were included in the study. Before each step of the procedure, the rabbits were anesthetized with intramuscular injections of ketamine HCl (30 mg/kg) and xylazine (5 mg/kg). The pupils of experimental eyes were dilated with phenylephrine (5.0%) and tropicamide (0.25%).

Vitreous Gas Compression and Gas–Fluid Exchange

The procedure of vitreous gas compression has been described elsewhere. Briefly, cryopexy was performed at the lower nasal quadrant at approximately 4 mm distance from the limbus to prevent the retinal detachment cause by subsequent intraocular procedures. Seven to 10 days later, 0.3 ml of perfluorocarbon gas was injected intravitreally with a 30-gauge tuberculin syringe in two portions of 0.2 ml and 0.1 ml under indirect ophthalmoscopic observation. The gas was left in the eye for 2 days. After this, the intraocular gas bubble nearly filled the vitreous cavity and was then replaced by Ringer’s solution.

Intravitreal Injection of Fibroblasts

For intravitreal injection, tissue-cultured homologous rabbit dermal fibroblasts up to the third passage were allowed to grow to confluence. The cultures were trypsinized for 4 min and then suspended in 25 ml of Dulbecco’s modified Eagle’s medium (with 10% bovine serum, penicillin sodium, streptomycin, and amphotericin B). The cell suspension was centrifuged at 900 rpm for 10 min. The medium was removed, and the cells were resuspended in 4 ml of Ca- and Mg-free phosphate-buffered saline (PBS). The cell number in a 0.1-ml aliquot was determined, and enough PBS was added to achieve a final cell density of 25,000/0.1 ml for intravitreal injection.

Seven to 10 days after gas–fluid exchange, photographic documentation of all experimental eyes was performed. Eyes with cataract, vitreous hemorrhage, or any degree of retinal detachment were excluded from the study. These animals were killed by injection of 0.5 ml of pentobarbital into the marginal ear vein. The remaining eyes received intraocular injections of 25,000 fibroblasts directly above the optic disc.

Efficacy of Aclacinomycin A

To provide sufficient time for the fibroblasts to adjust to the vitreous environment and start to proliferate, the drug was administered 2–3 days after the injection of cells. Before drug administration, all eyes were examined and documented by fundus drawings and photography. Those with vitreous hemorrhage or any degree of retinal detachment at this point in the study were excluded.

Twenty-four hours before the fibroblast injection, 39 eyes received intravitreal injections of 0.1 ml of PBS. This was done because these eyes were part of a larger study in which such an injection was part of the protocol. Three days after fibroblast injection, all
eyes were examined and photographed. Those with any degree of retinal detachment were excluded. The eyes were randomly assigned to three groups. Sixteen eyes served as controls and received intraocular injections of 0.15 ml of PBS subdivided in volumes of 0.1 and 0.05 ml given 4 hr apart. Eight eyes received 6 nmol of aclacinomycin A suspended in PBS (Ca- and Mg-free) subdivided into two doses of 4 nmol (in 0.1 ml of PBS) and 2 nmol (in 0.05 ml of PBS) 4 hr apart. Fifteen eyes received 60 nmol of aclacinomycin A subdivided into two doses of 40 and 20 nmol given 4 hr apart. The drug was administered in two doses because previous experiments using daunomycin had shown that single-dose injections were not effective when given on day 3.22

After 60 nmol of aclacinomycin A administered in subdivided doses 3 days after the fibroblast injection had been found to be effective to prevent PVR, 25 eyes were randomly assigned to two groups. Nine eyes served as controls and received single injections of 0.15 ml of PBS 2 days after the fibroblast injection. Sixteen eyes received 30 nmol of aclacinomycin A in 0.15 ml of PBS as a single dose 2 days after the fibroblast injection. No preinjection of PBS before the injection of cells was performed in this series.

Follow-Up
All eyes were followed by indirect ophthalmoscopy, fundus drawings, and photography on days 7, 14 and 28 after fibroblast injection. To determine the grade of detachment, the previously published staging system for experimental PVR in rabbits was used.23 These authors distinguished eight PVR stages, three of which describe detached retina. The unequivocal absence or presence of retinal detachment was used as an indicator of the success or failure of the treatment. The results were subjected to statistical analysis using Fisher’s two-tailed exact test. On day 28, all animals were killed by pentobarbital injection. The experimental eyes were enucleated and kept in fixative (2.5% glutaraldehyde and 2.0% paraformaldehyde) for morphologic evaluation.

Retinal Toxicity of Aclacinomycin A
To evaluate the potential toxic effects of 60 nmol of aclacinomycin A on the rabbit retina, nine rabbits were assigned to three groups after undergoing vitreous gas compression and gas–fluid exchange in both eyes. Ten days after the latter, all right eyes were injected with 60 nmol of aclacinomycin A subdivided into two doses of 40 and 20 nmol given 4 hr apart as described. All left eyes received sham injections (0.1 and 0.05 ml of PBS). Three additional eyes received single doses of 30 nmol of aclacinomycin A after being subjected to the same protocol.

ERG
On days 0, 3, 7, and 14 after drug administration, scotopic and photopic ERG were recorded from both eyes using a two-channel visual assessment device (Cadwell 5200, Kennewick, WA) and a Ganzfeld flash unit (Cadwell VPA-10). After dark adaptation for at least 20 min, corneal surface gold electrodes were attached to both eyes. The intradermal reference electrodes were short circuited. Twenty scotopic recordings were simultaneously obtained from both eyes by applying white stroboscope stimuli with maximum light intensity. These were electronically averaged. Photopic ERG were recorded in the same manner after 10 min for light adaptation. Two scotopic and photopic averages were obtained from each eye after switching active channels between both eyes. The ERG responses were evaluated using right–left ratios created by dividing the voltages obtained from drug-treated eyes by those used in control eyes. This allowed us to eliminate individual and daily variations.24-26 Differences between recordings were subjected to statistical analysis using one-way analysis of variance (t test).

Transmission Electron Microscopy
Three rabbits that received 60 nmol of aclacinomycin A were killed on days 3, 7, and 14 after drug administration, and all eyes were enucleated. On day 14, also all three eyes injected with 30 nmol of aclacinomycin A were enucleated. The eyes were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde. Two specimens were obtained from a central area below the optic nerve head containing retina, choroid, and sclera and were embedded in an epoxy resin.27 To avoid bias in taking and reading the micrographs, the specimens were masked.

RESULTS
Efficacy of Aclacinomycin A
Four of 15 eyes treated with 60 nmol of aclacinomycin A in subdivided doses of 40 and 20 nmol given 3 days after fibroblast injection developed retinal detachment by day 28 (detachment rate, 26.7%; P < 0.0001). Three detachments were Stage 5, and one was Stage 6 (Table 1). All eight eyes that had received 6 nmol of aclacinomycin A in subdivided doses of 4 and 2 nmol given 3 days after fibroblast injection developed complete traction detachment by day 28 (detachment rate, 100%). All 16 control eyes also had complete retinal detachments (detachment rate, 100%). Six of 16 eyes treated with a single dose of 30 nmol of aclacinomycin A given 2 days after fibroblast injection developed reti-
TABLE 1. Stages of Experimental Proliferative Vitreoretinopathy in Treated Eyes and Controls at Day 28

<table>
<thead>
<tr>
<th>Stage</th>
<th>Control*</th>
<th>Control†</th>
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<td>0</td>
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<td>6 nmol*</td>
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<tr>
<td>Total</td>
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Adapted from reference 23: attached retina, stages 0–4; detached retina, stages 5–7.
* Administered in subdivided doses on day 3.
† Administered in single doses on day 2.

Stages of Experimental Proliferative Vitreoretinopathy in Treated Eyes and Controls at Day 28 day (Fig. 4). Electron microscopic studies of eyes treated with 60 nmol of aclacinomycin A revealed no acute or late damage to any layer of the retina on any observation day (Fig. 5). Because eyes treated with 30 nmol of aclacinomycin A had normal ERG and those exposed to 60 nmol had a normal ultrastructure, no morphologic studies were conducted on the former.

DISCUSSION

Our experiments showed that intraocular administration of aclacinomycin A in doses of 30 nmol (as a single dose 2 days after the fibroblast injection) and 60 nmol (in divided doses 3 days after the fibroblast injection) were both effective in significantly reducing the incidence and severity of experimental PVR. Toxicity studies after administration of 60 nmol of aclacinomycin A in eyes having undergone vitreous gas compression but no fibroblast injection revealed only transient ERG changes. No significant ERG changes were observed after administration of 30 nmol. Transmission electron microscopic studies conducted on eyes injected with 60 nmol of this drug did not reveal drug-related damage to the retina on days 3, 7, or 14 after drug administration.

Effective doses were established in a rabbit model of PVR for daunomycin. However, we have reservations regarding its use because all doses found to be efficacious in experimental PVR were also shown to cause some toxic damage to the retina. No useful data on the potential retinal toxicity of daunomycin in treatment of human PVR have been published because it has been used in eyes with pre-existing damage. Various authors sought to mitigate the potential toxic effects of daunomycin by either administering divided doses or by combining...
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R/L-Ratio

Day 0 Day 3 Day 7 Day 14

a-wave ratio

b-wave ratio

Day 0 Day 3 Day 7 Day 14

R/L-Ratio

b-wave ratio

Day 0 Day 3 Day 7 Day 14

FIGURE 3. Right–left ratios in (A) scotopic and (B) photopic ERG after 60 nmol of aclacinomycin A. On day 3, a- and b-waves recorded from drug-treated eyes decreased transiently.

daunomycin with corticosteroids.21,22 One group compared the efficacy of single and divided doses in experimental PVR.22 They found that, 3 days after fibroblast injection, administration of divided doses of daunomycin given a few hours apart was effective; the same amount given as a single dose was not. Unfortunately, they did not provide data about the retinal toxicity of divided drug administration. The other group compared the efficacy of combined treatment with daunomycin and triamcinolone acetonide versus daunomycin alone.21 The rationale was that smaller doses of daunomycin might be given in combination therapy while maintaining sufficient efficacy. Because no significant difference was found between both treatment groups, they did not pursue this approach any further.

A second concern, which, to our knowledge, has not been addressed in the ophthalmic literature, is the high mutagenic and carcinogenic potential of daunomycin. Various investigators have reported carcinogenic effects of daunomycin observed in vitro and in animal models.11–13,15,18 Induction of malignant tumors has been published, even after a single administration of daunomycin.11 We think that intraocular administration of highly carcinogenic drugs might impose a risk for patients; this should be avoided if alternative noncarcinogenic agents are available. Pharmacologic studies have shown that N-alkylation of the anthracycline aminosugar moiety can result in a complete loss of carcinogenicity.14,16 Aclacinomycin A is a N-dimethylated oligosaccharide anthracycline that has been proved to be noncarcinogenic by numerous investigators.16–19,29–31 In several Phase II studies, aclacinomycin A has successfully been used in the treatment of malignant proliferative diseases.30,32–36

Aclacinomycin A has never been studied concerning its suitability for treatment of proliferative ocular disorders. There is only one report in which the local irritative effects were studied in the eye after instillation of a 1% solution into the inferior conjunctival fornix.37 Dilatation of blood vessels and swelling of the

FIGURE 4. Right–left ratios in (A) scotopic and (B) photopic ERG after 30 nmol of aclacinomycin A. The slightly declined scotopic b-wave ratio on day 3 was not significantly different from the pre-exposure ratio on day 0 (P = 0.065, by t test).
FIGURE 5. Transmission electron micrographs of the well-preserved photoreceptors and the inner nuclear layer after administration of 60 nmol of actinomycin A (A, B) and after sham treatment (C, D) (on day 7).
conjunctiva and nictitating membrane were found. Histologic studies showed edema of the conjunctiva, cornea, and iris, all of which could be avoided by thoroughly irrigating the eye surface immediately after drug application.

In summary, our study demonstrated that aclacinomycin A effectively reduced the frequency and severity of retinal traction detachment in experimental PVR. The highest effective dose used in the study caused only transient ERG changes; these completely recovered. No acute or late morphologic damage to the retina could be observed. Therefore, we suggest aclacinomycin A as an alternative to daunomycin for further evaluation of its suitability for the treatment of human PVR.

Key Words
aclacinomycin A, daunomycin, anthracyclines, proliferative vitreoretinopathy, PVR

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References
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