The Effect of Combined Daunorubicin and Triamcinolone Acetonide Treatment on a Refined Experimental Model of Proliferative Vitreoretinopathy

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Prior studies have shown that intravitreal daunorubicin (9–15 nmol) and triamcinolone acetonide (2 mg) are effective individually in preventing retinal detachment in experimental proliferative vitreoretinopathy. This report compares the efficacy of the combination of daunorubicin (15 nmol) and triamcinolone acetonide (2 mg) with that of daunorubicin alone in a refined experimental model of proliferative vitreoretinopathy. The degree of retinal detachment in each treatment group was graded, with the unequivocal absence or presence of retinal detachment used as an indicator of treatment success or failure. Both treatments (daunorubicin alone and in combination with triamcinolone acetonide) effectively prevented retinal detachment. However, there was no significant difference in the rate of retinal detachment between the two treatment groups. These results indicate that combination therapy with daunorubicin/triamcinolone is no more effective at preventing retinal detachment than daunorubicin alone. Invest Ophthalmol Vis Sci 33:2160–2164, 1992

Proliferative vitreoretinopathy (PVR) is a major complication of retinal surgery. This condition is characterized by cellular proliferation on the anterior and posterior surfaces of the retina and along formed vitreous, resulting in the formation of strands and membranes as well as fixed retinal folding.1,2 Mechanical removal of these membranes allows for the treatment of these diseases. However, the clinician often is faced with the recurrence of proliferation.

Animal models have been developed that can reproduce many of the features of PVR.3–8 One such model, the refined experimental model of proliferative vitreoretinopathy, involves the injection of 25,000 fibroblasts into an eye that has previously undergone gas-compression vitrectomy.9 We showed that 15 nmol daunorubicin in this model resulted in a reduction of retinal detachment from 100% (control) to 25% (treated) when injected in divided doses 3 d after cell injection.9 Corticosteroids alone also are efficacious in preventing retinal detachment in this model, but to a lesser degree. When injected 24 hr prior to cell injection, 2 mg triamcinolone acetonide reduced the rate of retinal detachment from 85% (control) to 43% (treated).10

The purpose of this study was to determine if combination treatment with daunorubicin and triamcinolone acetonide would be more efficacious than treatment with daunorubicin alone in preventing retinal detachment. If so, it might be possible to decrease the concentration of daunorubicin or triamcinolone acetonide to reduce the chance of retinal toxicity. Preliminary results were presented at the May 1991 ARVO meeting in Sarasota, Florida.

Materials and Methods

Preparation of Cultured Fibroblasts

Dermal fibroblasts were obtained from rabbit rump explants, prepared under sterile conditions and incubated in 75 cm² culture flasks (Corning, Wexford, PA). Primary cultures were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, antibiotics (penicillin sodium, streptomycin sulfate), and antimycotics (amphotericin B) under a humidified atmosphere of 5% carbon dioxide in air. Cells were harvested by incubating them with 3.5 ml of 0.04% trypsin for 4 min and collecting the cells in stop media (Dulbecco's modified Eagle's medium with 10% calf serum plus antibiotics and antimycotics). The dispersed cells were centrifuged at 900 rpm
for 10 min and resuspended in 4 ml of phosphate-buffered saline (PBS). The cell count in a 0.1 ml aliquot was determined and enough PBS was added to achieve a final concentration of 25,000 per 0.1 ml in the remainder of the suspension.

Gas Compression Vitrectomy and Gas-Fluid Exchange

Fifty Dutch-belted pigmented rabbits of both sexes weighing 2.5–5.0 pounds were anesthetized with 0.5 ml ketamine and 0.1 ml xylazine. Their pupils were dilated with 5.0% phenylephrine and 0.25% tropicamide prior to all procedures and photographs. All experimental procedures conformed to the ARVO Resolution of the Use of Animals in Research.

The technique of gas-mediated vitreous compression as part of the refined experimental model of PVR has been previously described.8,11 Transcleral cryolesions were placed to create an area where repeated injections could be made. Ten days after cryopexy, perfluoropropane gas (0.4 ml) was injected. Two days later, the gas had expanded to fill 80%–90% of the vitreous cavity. A syringe with a 30 G needle was inserted inferiorly, and approximately 0.2–0.3 ml of sterile Ringer’s lactate solution was injected. Gas escaped readily from the hole when the needle was withdrawn. This process of slow injection and gas escape was repeated until gas could no longer be seen in the eye. The entry site then was closed with a preplaced 7-0 Vicryl (Ethicon, Somerville, NJ) suture.

Intravitreal Injections of Fibroblasts, Daunorubicin, and Triamcinolone Acetonide.

Ten days after gas-fluid exchange, the eye was propo- sed and a 27 G needle was inserted through the sclera and retina 4 mm posterior to the corneoscleral junction in the superotemporal quadrant under stereomicroscopic control. With the bevel of the needle directed downward, 25,000 tissue-cultured homologous dermal fibroblasts suspended in 0.1 ml sterile PBS were injected over the optic nerve and medullary rays. The animals were immediately placed on their backs for 1 hr to allow the cells to settle over the vascular wings of the retina.8

The rabbits then were randomized into three groups. Group 1 (n = 18) received one intravitreal injection of triamcinolone acetonide, 2 mg/0.1 ml 0.005% tyloxapol, 24 hr prior to cell injection and 10 nmol daunorubicin/0.1 ml PBS followed by a second injection of 5 nmol daunorubicin/0.05 ml PBS 4 hr thereafter on the third day after cell injection. Group 2 (n = 16) received daunorubicin at the same dosages and times as well as the triamcinolone vehicle. Group 3 (n = 16) received both drug vehicles. Injection of the drugs and vehicles was performed identically to that of fibroblast injection.

Clinical Examination

Each eye was examined by indirect ophthalmoscopy and followed by fundus drawings and fundus photography on days 0, 3, 7, 14, and 28 after fibroblast injection. All rabbits were killed on day 28 with an overdose of 1.0 ml pentobarbital sodium via ear vein infusion.

Grading of Proliferative Vitreoretinopathy

The progression of proliferative vitreoretinopathy was graded using the classification system of Hida et al.12 Stage 0 showed no abnormalities in the fundus. Stage 1 showed surface wrinkling of the medullary wing, resulting in a beaten metal appearance. Stage 2 showed mild puckering and small focal contractions on the nonelevated wings. Stage 3 showed severe puckering and contraction of the whole medullary wings. Stage 4 showed elevated puckering caused by a vitreous strand. Stage 5 showed partial retinal detachment involving one medullary wing. Stage 6 showed low retinal detachment involving both wings with the avascular retina attached. Stage 7 showed total retinal detachment with a closed funnel appearance. The unequivocal absence (grades 0–4) or presence (grades 5–7) of retinal detachment was used as the indicator of success or failure of combination therapy and treatment with daunorubicin alone. The results were subjected to statistical analysis using the Fisher’s exact test.

Observation of Neovascularization

All eyes that progressed to stage 7 retinal detachment were observed and documented for the presence of concomitant neovascularization.

Results

Proliferative Vitreoretinopathy

All animals receiving only drug vehicles (n = 16) developed stage 7 retinal detachment (100%) by day 28 (Figure 1). Seven of the 16 animals receiving daunorubicin alone developed retinal detachment (43.8%) by day 28. Of these failures, two eyes were stage 5, two were stage 6, and three were stage 7. Five of the 18 animals receiving both daunorubicin and triamcinolone acetonide developed retinal detachments (27.8%) by day 28. Of these failures, two eyes had stage 5 detachments, one had a stage 6 detachment, and two eyes had stage 7 detachments. The overall distribution of final stage for all eyes at day 28 is shown in Table 1.
The rate of retinal detachment at day 28 was significantly less in both treated groups compared to controls \( (P < 0.0001, \text{Fisher's exact test}) \). There was no significant difference between the experimental groups in prevention of proliferative vitreoretinopathy \( (\text{risk ratio} = 13.9; 95\% \text{ confidence interval} = 0.58-3.29) \).

Neovascularization

Twelve of the 16 stage 7 control eyes developed concomitant neovascularization as well. All three of the stage 7 eyes in the daunorubicin group developed neovascularization \( (100\%) \), whereas neither stage 7 eye in the combination therapy group developed neovascularization \( (\text{Figures 2, 3}) \). Statistical analysis was not performed on these observations because of the small number of rabbits with stage 7 eyes.

Discussion

Although treatment with daunorubicin alone and in combination with triamcinolone acetonide led to a significant decrease in the rate of retinal detachment in experimental proliferative vitreoretinopathy compared to controls, combination treatment with daunorubicin and triamcinolone acetonide was shown to be no more effective than treatment with daunorubicin alone. There was no statistically significant difference between the treatment groups regarding the rate of retinal detachment \( (43.8\% \text{ in the daunorubicin group vs. } 27.8\% \text{ in the combination group}) \). The 95% confidence interval for the difference between these two proportions is \( 16 \pm 32\% \) \( (\text{mean} \pm \text{standard error of the mean}) \). This width indicates that our experiment did not have the precision to detect subtle differences in outcome between these two groups (ie, we could detect differences in the order of 28% vs. 60%, which is 32% on the absolute scale, but we could detect no smaller differences). We would have needed approximately 60 rabbits per group to have had the statistical power to detect a difference in response rates on the order of 44% versus 28%. Because this was prohibitively expensive, we stopped the experiment. It is disappointing that our results did not show better efficacy with the drugs in combination. We had hoped to be able to next decrease the concentration of daunorubicin because daunorubicin has a low therapeutic index. Wiedemann et al\(^{13}\) found that doses above 27 nmol were toxic to the retina, as evidenced by the presence of retinal holes and retinal detachments. Santana et al\(^{14}\) demonstrated photoreceptor outer segment damage, by light and electron microscopy, in dosages as low as 15 nmol, whereas even at the therapeutic dose of 9 nmol, outer segment abnormalities were observed by electron microscopy. Triamcinolone acetonide \( (1 \text{ mg}) \), on the other hand, does not cause ocular toxic effects as assessed by slit-lamp examinations, ophthalmoscopy, light and electron microscopy, electroretinography, or intraocular pressure measurements.\(^{13}\)

Many investigators previously have proven daunorubicin\(^{5,13,16}\) and triamcinolone acetonide\(^{10,17,18}\) to be individually effective in preventing retinal detachment in experimental models of proliferative vitreoretinopathy. However, no studies have examined the efficacy of a combination of these two agents. The purpose of this experiment was to combine daunorubicin with a substance known to be less toxic to the retina and with a different mechanism of action to further decrease the rates of retinal detachment.

The cytostatic effects of daunorubicin, an anthracycline antibiotic, have been proposed to depend on a number of mechanisms involving DNA binding, free radical formation, ion-pump inhibition, metal ion chelation, and cell membrane disruption.\(^{19,20}\) Preferential binding of these agents to DNA instead of RNA

Table 1. Stages of proliferative vitreoretinopathy of Hida et al.\(^{12}\) in control and treated eyes on day 28 (number of eyes)

<table>
<thead>
<tr>
<th>Stage (Hida et al.(^{12}))</th>
<th>Control</th>
<th>Daunorubicin alone</th>
<th>Daunorubicin and triamcinolone acetonide</th>
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also has been shown to lead to mutagenic, teratogenic, and carcinogenic effects in living organisms.

Daunorubicin has been proven to be efficacious in preventing retinal detachment in experimental proliferative vitreoretinopathy. Wiedemann et al. demonstrated that a dose of 9 nmol per eye reduced the incidence of retinal detachment in experimental proliferative vitreoretinopathy from the 75% seen in controls to 25% after 28 d, whereas doses between 5 and 15 nmol per eye were effective in preventing retinal detachment. In the refined experimental model of proliferative vitreoretinopathy, Khawly et al. showed that daunorubicin, in divided doses of 10 nmol followed by 5 nmol 4 hr later on the third day after cell injection, reduced the rate of retinal detachment from 100% to 25%. Daunorubicin was not effective as a single 15 nmol dose. In this case, the rate of retinal detachment was reduced from 100% to 73%.

Steroids exert their antiproliferative effect by inhibiting DNA, RNA, and protein synthesis as well as altering cell membrane permeability. Triamcinolone acetonide inhibits fibroblast growth and mitotic activity in cells in tissue culture. Tano et al. injected 250,000 fibroblasts into rabbit eyes and found that simultaneous injection of 1 mg triamcinolone acetonide reduced the percentage of traction retinal detachment from 84% to 20% and the percentage of neovascularization from 58% to 0% in eyes receiving triamcinolone acetonide compared with eyes receiving only the drug vehicle. Chandler et al. reduced the rate of retinal detachment from 90% to 56% with 2 mg triamcinolone acetonide in the refined experimental model of proliferative vitreoretinopathy. An improvement in the efficacy in the same model was demonstrated when the drug was injected 24 hr prior to cell injection. Antoszyk et al. later demonstrated that in an experimental model of preretinal neovascularization in the rabbit, pretreatment with 2 mg triamcinolone acetonide reduced the incidence of severe neovascularization from 100% to 14%, whereas treatment with 2 mg triamcinolone acetonide 3 d after hyaluronidase and cell injection reduced the rate of severe neovascularization from 100% to 54%.

The rate of retinal detachment in eyes receiving daunorubicin alone was found to be 43.8% in our experiments. This rate is slightly higher than that shown by Khawly et al., who demonstrated a 25% rate of retinal detachment in groups treated with daunorubicin. This discrepancy may be explained by a difference in the experimental protocol. To maintain identical conditions among all experimental groups, it was necessary for us to inject the daunorubicin group with the triamcinolone acetonide vehicle 24 hr prior to cell injection so a valid comparison between the two treatment groups could be made. This extra injection constitutes an additional breakdown of the blood-retinal barrier. Thus, conditions in the two experiments were not the same.

Although not the main focus of the present investigation, we were curious about whether triamcinolone acetonide would decrease the rate of neovascularization in stage 7 retinal detachments, as shown in previous reports. We observed that in eyes receiving triamcinolone acetonide (ie, the combination group), there was no evidence of neovascularization in eyes with stage 7 retinal detachment, whereas in eyes that did not receive triamcinolone acetonide, neovascularization was observed in the majority of eyes with stage...
7 retinal detachment (all three eyes in the daunorubicin group and 12 of the 16 control eyes). Because we did not perform statistical analysis on this data, these results do not confirm previous investigations by Antoszyk et al. but are consistent with them.25–27

Key words: daunorubicin, triamcinolone acetonide, proliferative vitreoretinopathy, PVR, retina, vitreous.

References