Laboratory investigations

Graefe's Archive for Clinical and Experimental Ophthalmology

Graefe's Arch Clin Exp Ophthalmol (1990) 228:513–516

Taxol treatment of experimental proliferative vitreoretinopathy

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Received April 27, 1989 / Accepted February 14, 1990

Abstract. Taxol is a potent stabilizer of microtubules, and inhibitor of in vitro replication, migration, and contraction of fibroblasts. It has been found to limit the development of experimental tractional retinal detachments in nonvitrectomized rabbit eyes. We used taxol in vitrectomized, phakic rabbit eyes with experimentally induced proliferative vitreoretinopathy and tractional retinal detachments. Taxol was dissolved in 30% DMSO because of poor aqueous solubility. A single 0.1 ml intravitreal dose of 2×10^{-4} *M* taxol in 30% DMSO was injected immediately after 250000 heterologous corneal fibroblasts had been injected; 0.1 ml of 30% DMSO was injected into control eyes. Taxol reduced the incidence of tractional retinal detachments seen 3–4 weeks later. When taxol injection was delayed for 3 days after the initial intravitreal injection of fibroblasts into nonvitrectomized eyes, the extent of retinal detachments was reduced, but the incidence of retinal detachment was unchanged from the untreated eyes at the end of 4 weeks. These data indicate that taxol may be most useful when given early in the course of proliferative vitreoretinopathy.

Introduction

Proliferative vitreoretinopathy (PVR) is the term proposed by the Retina Society to describe the clinical condition characterized by the proliferation of cells derived from the retinal pigment epithelium, retinal glia, and fibroblasts on vitreoretinal surfaces [9]. The resultant membrane formation can distort the retina and exert tractional forces which may cause retinal detachment and retinal tears [5]. PVR is the most common cause of failure in 5%–10% of retinal reattachment operations [9].

Dexamethasone [16], triamcinolone [15], colchicine [8], 5-fluorouracil [2, 3, 13], actinomycin C, daunomycin, and doxorubicin [7] have been studied experimentally to control the development of PVR. Taxol prevents fibroblast migration behavior and replication by stabilizing microtubles and blocking the G2 and M phase of the cell cycle [1, 11]. Taxol has been found to effectively control the developments of retinal detachments in rabbit eyes with experimentally induced PVR [18].

The surgical treatment of retinal detachments complicated by the advanced grades of PVR is difficult and may require vitrectomy in addition to the conventional scleral buckling techniques [10]. Vitrectomy itself may exacerbate the proliferation of the intraocular cellular matrix responsible for PVR [6]. We have studied the efficacy of taxol in controlling experimentally induced PVR in vitrectomized and nonvitrectomized rabbit eyes and have determined the effect of taxol in controlling the development of PVR when delayed for 3 days after the intravitreal inoculation of fibroblasts.

Materials and methods

Twent-two adult New Zealand pigmented rabbits of both sexes with an average weight of 3 kg each, were used in this study. The rabbits were divided into two groups. In group I, comprised of 12 rabbits: (1) all rabbits underwent bilateral vitrectomy; (2) all eyes received an intravitreal inoculum of 250000 fibroblasts; (3) 12 randomly selected eyes were treated with intravitreal taxol in 30% DMSO; (4) intravitreal 30% DMSO in BSS (Alcon Laboratories, Fort Worth, Tex.) was injected into the remaining 12 eyes as controls.

Prior to vitrectomy, the rabbits in group I were anesthetized with ketamine HCl (15 mg/kg body weight) and acepromazine (5 mg/kg body weight). Retrobulbar anesthesia was achieved with the injection of 1 ml of 2% xylocaine. The nictitating membrane was removed and a lateral canthotomy was performed. Pupillary dilation was achieved with 10% phenylephrine, 1% tropicamide, and 1% atropine sulfate. After performing a peritomy, a sclerotomy was made 2 mm posterior to the limbus in the middle of each of the two superior quadrants of the globe with a microvitreoretinal (MVR) blade.

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Vitrectomy was performed with an Ocutome (Cooper Vision, Irvine, Calif.), illuminated with a coaxial operating microscope and an 8-mm flat contact lens. We injected 0.1 ml of 1% sodium fluorescein into the vitreous body to enhance visualization during vitrectomy. After a core vitrectomy was performed, the sclerotomy sites were closed with 10-0 nylon sutures. Atropine ointment and polymyxin B-neomycin-bacitracin ointment were applied to the palpebral fissure postoperatively and the eyelids taped shut. One week after the vitrectomy, the eyes were examined for retinal breaks or detachments on which basis rabbits were immediately excluded from the study.

Rabbit corneal fibroblasts were obtained from the American Type Culture Collection, (Rockville, Md.). Monolayer cell cultures of rabbit corneal fibroblasts were grown in minimal essential medium supplemented with 10% fetal bovine serum in a 37° C, 5% carbon dioxide, humidified incubator. Confluent growth occurred at 96 h. At this time, the cells were harvested by washing the cultures with Ca^{2+} and Mg^{2+} free phosphate-buffered saline, trypsinized (0.25% trypsin and 0.02% EDTA), dispersed, centrifuged, resuspended in culture media, and cultures in T-25 culture flasks. The cells were washed, trypsinized, dispersed, and resuspended in balanced salt solution (BSS). Aliquots were removed to determine the cell concentration by hemocytometer. The suspended cells were diluted to a concentration of 250000 cells/0.1 ml. Cell viability was determined by the tryphan blue exclusion test and found to be greater than 94% before intravitreal injection.

Taxol was obtained from the National Cancer Institute, dissolved in 30% DMSO, and stored at −20° C. The intravitreal injection dose was 0.1 ml of 2×10^{-4} *M* taxol delivered via a tuberculin syringe with a 5/8 inch no. 26 needle.

One week after the vitrectomy, the rabbits were anesthetized with ketamine and acepromazine as described previously. Topical 0.5% proparacaine anesthetic eye drops were also used during the procedure. An anterior chamber paracentesis was performed with a 5/8 inch no. 26 needle in order to maintain normal ocular tonus. An inoculum of 250000 cells suspended in 0.1 ml BSS in a tuberculin syringe with a 5/8 inch no. 26 needle was injected into the midvitreous space through the superior temporal sclerotomy site of the previously performed vitrectomy. Immediately following the injection, 0.1 ml of 2×10^{-4} *M* taxol in a tuberculin syringe with a no. 26 needle was injected into the midvitreous cavity through the same site. The control eyes received an intravitreal injection of 0.1 ml of 30% DMSO in BSS.

In group II, comprised of 10 rabbits: (1) no vitrectomies were performed; (2) all eyes received an intravitreal inoculum of 250000 fibroblasts; (3) in ten randomly selected eyes, intravitreal injection of taxol in 30% DMSO was delayed for 3 days after inoculation of fibroblasts; (4) intravitreal 30% DMSO in BSS was injected into the remaining ten eyes as controls.

We examined the animals at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after the intravitreal injection of fibroblasts. The observers were masked as to the eyes treated with taxol. Two examiners were present to obtain concurrent observations. Fundus photographs were obtained in selected cases. A retinal drawing was made by the observers at each examination. We classified the degree of PVR according to the schema of Fastenberg et al. [5]: stage 1, intravitreal membrane; stage 2, focal traction, localized vascular changes, and blood vessel elevation; stage 3, localized detachment, generally of the medullary rays, or localized pucker; stage 4, extensive retinal detachment, total medullary ray detachment, or peripapillary retinal detachment; stage 5, total retinal detachment, retinal folds and hole formation.

The data obtained from both groups I and II were analyzed by McNemar's test of paired alternatives to determine statistical significance [14].

Results

In group I, 1 week after intravitreal inoculation of the fibroblasts, 2/12 or 17% of the eyes treated with taxol developed tractional retinal detachment while 3/12 or 25% of the eyes without taxol developed similar changes.

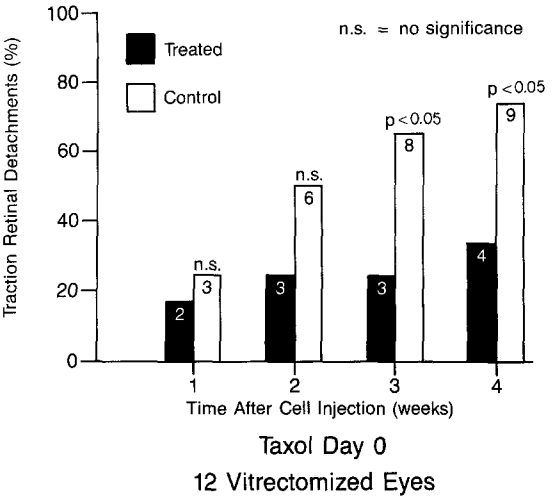

Fig. 1. Incidence of retinal detachment with immediate taxol injection

Two weeks after intravitreal inoculation of fibroblasts 3/12 or 25% of taxol treated eyes developed tractional retinal detachments while 6/12 or 50% of control eyes developed similar changes. Three weeks after inoculation with intraviteal fibroblasts, 3/12 or 25% of taxol treated eyes developed tractional retinal detachments, but 8/12 or 67% of control eyes developed these changes. Four weeks after inoculation with intravitreal fibroblasts, 4/12 or 33% of the taxol treated eyes developed tractional retinal detachments, and 9/12 or 75% of control eyes developed similar changes. There was a significant ($P<0.05$) difference at 3 weeks and 4 weeks between the taxol treated eyes and the untreated control eyes. These data are summarized in Fig. 1.

The extent of retinal detachment from PVR was graded according to the schema of Fastenberg et al. which ranged between stage 1 and stage 5 [5]. By assigning a point for each stage of retinal detachment to grade the extent of the PVR, the taxol treated eyes were found to average stage 1.4 PVR while the control eyes averaged stage 2.0 PVR at 1 week after inoculation with intravitreal fibroblasts. Two weeks after inoculation, taxol treated eyes averaged stage 1.5 PVR while untreated control eyes averaged stage 2.6 PVR. Three weeks after inoculation, taxol treated eyes averaged stage 1.6 PVR and untreated control eyes averaged stage 3.3 PVR. At 4 weeks after inoculation, taxol treated eyes averaged stage 1.6 PVR while untreated control eyes averaged stage 3.7 PVR. There was a significant difference in the extent of PVR at 2 weeks ($P<0.01$), 3 weeks ($P<0.01$), and 4 weeks ($P<0.001$) after fibroblast inoculation between the taxol treated eyes and control eyes. These data are summarized in Fig. 2.

In group II, 2/10 or 20% of taxol treated eyes developed retinal detachments while 5/10 or 50% of control eyes developed retinal detachments 1 week after taxol

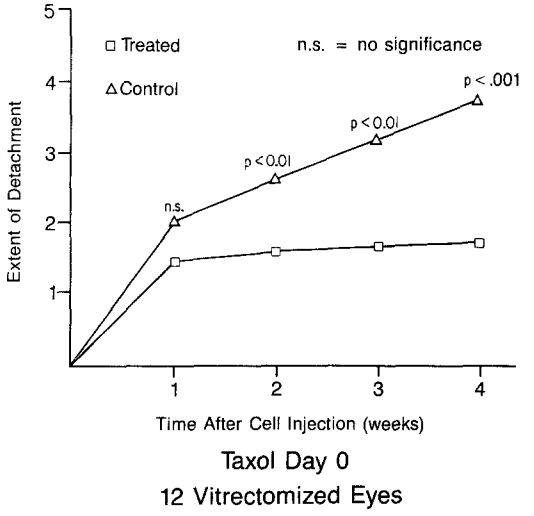

Fig. 2. Extent of retinal detachment with immediate taxol injection

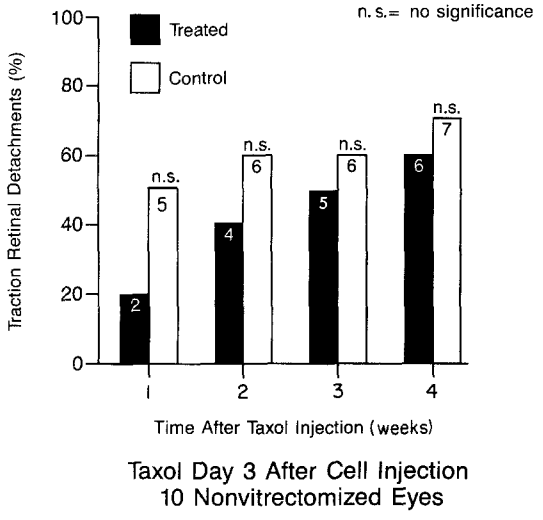

Fig. 3. Incidence of retinal detachment when taxol treatment was delayed for three days

treatment. Two weeks after taxol treatment, 4/10 or 40% of taxol treated eyes developed retinal detachment while 6/10 of 60% of control eyes developed retinal detachments. Three weeks after taxol treatment, 5/10 or 50% of taxol treated eyes had tractional retinal detachments while 6/10 or 60% of control eyes had similar changes. Four weeks after taxol treatment, 6/10 or 60% of taxol treated eyes developed tractional retinal detachments while 7/10 or 70% of control eyes had such changes. There were no significant differences in the incidence of retinal detachments between taxol treated eyes and control eyes at any time from 1 through 4 weeks after taxol treatment. These data are summarized in Fig. 3.

The extent of retinal detachment from PVR was analyzed using the schema of Fastenberg et al. [5] described above. At 1 week after taxol treatment, the taxol treated

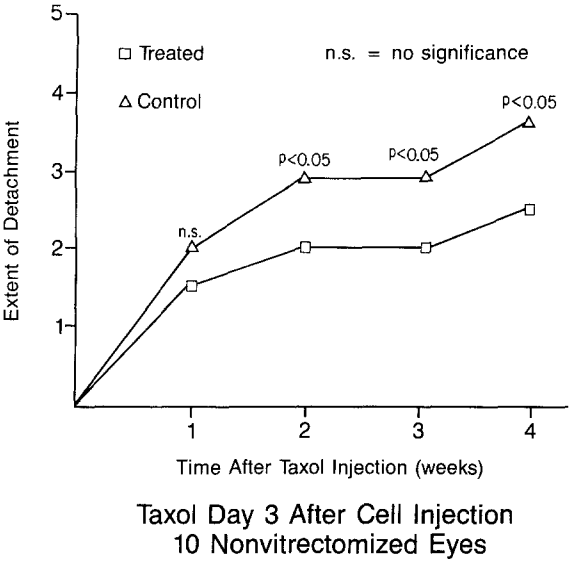

Fig. 4. Extent of retinal detachment when taxol was delayed for three days

eyes averaged stage 1.7 PVR while the control eyes averaged stage 2.0 PVR. At 2 weeks and again at 3 weeks after taxol treatment, the taxol treated eyes averaged stage 2.0 PVR while untreated control eyes averaged stage 2.8 PVR. Four weeks after taxol treatment, the taxol treated eyes averaged a stage 2.7 PVR while control eyes averaged a 3.7 stage of retinal detachment. There was a significant difference ($P < 0.05$) in the extent of retinal detachments from PVR between the taxol treated eyes versus controls from the 2nd week through the 4th week after taxol treatment. These data are summarized in Fig. 4.

Discussion

Proliferative vitreoretinopathy is a manifestation of cellular proliferation, attachment, and contraction on the vitreoretinal surfaces which often results in tractional and rhegmatogenous retinal detachments. Several drugs have been used to control PVR by inhibiting cellular proliferation, contraction, or collagen cross-linking. Antiproliferative agents such as 5-fluorouracil [2, 3, 13], daunomycin, and other drugs [7] have been found to be effective in controlling the development of PVR.

Taxol is isolated from the plant *Taxus brevifolia* and is characterised as an experimental antitumor agent [19]. Taxol is a stabilizer of microtubules and completely inhibits the replication, migration, and contraction of fibroblasts [11]. Cells are blocked in the G_2 and M phase of proliferation [1]. Taxol treated fibroblasts fail to make a normal mitotic apparatus even though they lose their nuclear membranes and condense their DNA. Intracellular microtubular complexes are seen in cultured cells for at least 48 h after incubation with taxol [17].

Taxol is poorly soluble in an aqueous medium and DMSO was used to achieve the concentrations necessary

for inhibition of PVR. DMSO in concentrations as high as 100% has been proposed as a vehicle for intravitreal drug injections and has been found to have no long-term retinal toxicity when used as a single intravitreal dose [12].

We have demonstrated that taxol is capable of reducing, but not eliminating retinal detachments due to experimentally induced PVR in vitrectomized rabbit eyes when administered immediately after an intravitreal inoculum of 250000 fibroblasts as described by Fastenberg et al. [5]. Van Bockxmeer et al. have reported the virtual elimination of retinal detachment with the use of intravitreal taxol injections in aphakic rabbit eyes inoculated with fibroblasts [18]. The higher rate of retinal detachments seen in our study may have several explanations. An increased proliferative response has been reported to occur after vitrectomy [6]. Vitrectomy may increase the clearance of intravitreal drugs and thereby reduce the time and dose of taxol available at the intraocular locus.

When taxol injection was delayed for 3 days or 72 h after the inoculation of fibroblasts in eyes which had not undergone vitrectomy, there was no significant difference in the incidence of retinal detachments between taxol treated eyes and untreated control eyes at all periods of observation up through 4 weeks after intravitreal taxol injection (Fig. 3). However, our studies did show that the extent of retinal detachments was significantly more severe in untreated control eyes from the 2nd through the 4th week after fibroblast inoculation. These results differ from those reported by van Bockxmeer et al. who administered taxol 36 h after the intravitreal inoculation of 250000 cells in rabbit eyes [18]. After 2 months of observation, they reported no progression of retinal detachments beyond 7 days after taxol treatment. We interpret this difference to mean that after a period of 3 days or 72 h, the proliferation of cellular elements has progressed to a point where even with taxol treatment, the population of cells which causes a tractional retinal detachment has been reached and far outweighs the inhibition of cellular migration or contraction. Van Bockxmeer et al. showed that there was significant inhibition of tractional retinal detachments even when the taxol administration was delayed for 36 h; however, they reported that one eye had developed grade 3 retinal detachment by day 7 [18]. We delayed the administration of taxol for 72 h. The doubling time of eukaryotic cells, is approximately 10–20 h [4]. A delay of another 36 h beyond the delay of 36 h in the van Bockxmeer study will allow another two or three generations to proliferate or a four- to eightfold increase of the original fibroblast population. Fastenberg et al. have clearly shown that the speed and severity of tractional retinal detachment in proliferative vitreoretinopathy is directly related to the number or population of fibroblasts injected into the vitreous body of rabbits [5]. Our work in comparison to the van Bockxmeer study clearly shows that a delay of 72 h may allow the fibroblast population to reach a point at which the contraction of existing fibroblast population becomes the dominant force in causing retinal detachments.

Our study suggests that a drug such as taxol which inhibits the replication, mobility, and contractility of fibroblasts must be given immediately or within 24–36 h after the development of retinal detachment. Treatment with taxol after this period of rapid cellular proliferation may no longer be effective in preventing retinal detachments due to PVR.

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