Eye (2007) 21, 784–788

www.nature.com/eye

CLINICAL STUDY

Evaluation of efficacy and safety of daunorubicin in glaucoma filtering surgery

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Abstract

Purpose **To evaluate the intraocular pressure (IOP)-lowering effect and ocular side effects of daunorubicin (DNR) in drug-modulated trabeculectomy.**

Methods **A prospective pilot study was conducted in which 21 Asian patients of Indian origin with high risk factors for failure of trabeculectomy were recruited. An approval from research ethics committee and an informed consent from every patient included in the study were obtained. DNR, an antimetabolite with known antifibroblastic action was used intraoperatively. A limbus-based conjunctival flap followed by conventional trabeculectomy was performed in all eyes. During trabeculectomy, a cellulose sponge soaked in 0.2 mg/ml DNR was applied for 3 min at the proposed site of trabeculectomy before preparation of the partial thickness scleral flap. A regular follow-up was carried out for 1 year where parameters including visual acuity, IOP by applanation tonometry, and slit lamp examination were performed on each visit. An IOP of 21 mmHg or less was taken as criteria for success.**

Results **Following DNR trabeculectomy, the IOP was lowered from baseline preoperative value of 36.19 ± 5.9 to 16.05 ± 2.52 mmHg at the end of 1 year. Success rate of 81% (17 out of 21 patients) was noted. None of the patients developed corneal epithelial toxicity, hypotony maculopathy, or choroidal detachment.**

Conclusion **The authors feel that intraoperative daunorubicin is safe and effective in lowering IOP in high-risk surgical cases of glaucoma. However, a much larger cohort study over a considerable number of years will eventually demonstrate its safety.**

Eye (2007) **21,** 784–788; doi:10.1038/sj.eye.6702328; published online 7 April 2006

Keywords: daunorubicin; glaucoma; anti-fibroblastic; trabeculectomy; intraocular pressure; high-risk factors

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Received: 15 August 2005
Accepted in revised form: 29 January 2006
Published online: 7 April 2006

This paper was presented as poster at the Royal College of Ophthalmologist annual meeting in Manchester 2004

Introduction

Conventional trabeculectomy, first time introduced by Cairn[1] is the most widely used glaucoma filtering surgery (GFS). Various authors have reported success rates varying from 75 to 90% in cases of primary glaucoma. However, success rate of trabeculectomy when evaluated in the presence of risk factors for the failure of GFS ranges from 30 to 60%.[2–5] The most common cause of failure of a GFS is the fibrosis at the conjunctival–episcleral interface. Various pharmacological agents that modulate wound healing by their antifibroblastic action have been used pre-, intra-, and postoperatively to improve the success rate in high-risk surgical cases of glaucoma. Most popular among these pharmacological agents are antimetabolites such as mitomycin C (MMC) and 5-fluorouracil (5-FU). However, use of these agents is associated with side effects, and new agents with antifibroblastic action are being constantly evaluated in an attempt to improve the success rate of GFS. Daunorubicin (DNR) is a glycosidic antimetabolite that inhibits fibroblast proliferation *in vitro* and *in vivo*.[6] The antifibroblastic action of DNR has been used both in animal studies and in human subjects to improve the success rate of various ophthalmic surgeries, including strabismus surgery,[7] pterygium surgery,[8] and retinal detachment surgery,[9] and in the prevention of posterior capsular opacification (PCO) following cataract extraction.[10,11] In glaucoma, DNR has been used experimentally and in human subjects to improve the success rate of high-risk GFS by way of a biodegradable slow-release subconjunctival (s/c) implant, as preoperative

and postoperative s/c injections following trabeculectomy.[12–15] In our study, we evaluated the efficacy and safety of intraoperative use of DNR in drug-modulated trabeculectomy. To the best of our knowledge, this antimetabolite has not been used intraoperatively in GFS before.

Patients and methods

A prospective study was conducted at Dr RP Centre for ophthalmic sciences (All India Institute of Medical Sciences) in which 21 Asian patients of Indian origin with inadequately controlled glaucoma and having high-risk factors that adversely affect the outcome of GFS were recruited. An approval from local research ethics committee and a written informed consent was taken from all patients participating in the study.

Case selection

The selection of the patients was based on a detailed history, systemic and ocular examination including visual acuity (VA), slit-lamp examination of anterior and posterior segment, optic disc evaluation using 90 D and direct ophthalmoscopy, intralocular pressure (IOP) measurement using applanation tonometer, and automated perimetry (where possible) . All patients included in this pilot study had high-risk factors reported in literature for failure of GFS[2–5] (Table 1). The youngest patient in the series was an infant with advanced congenital glaucoma who had a previously failed primary trabeculotomy.

Preparation of drug (DNR) for topical use

Each vial containing 20 mg DNR was diluted with 10 ml of normal saline, thus making a concentration of 2 mg/ml. From this solution 0.1 ml was then withdrawn in a sterile tuberculin syringe under aseptic precautions and 0.9 ml of normal saline was added to make a concentration of 0.2 mg/ml.

Table 1 Diagnosis of patients undergoing DNR trabeculectomy

Diagnosis of patients	*Number of patients (n = 21)*
Sturge–Weber syndrome and secondary glaucoma	2
Congenital glaucoma	1
Aphakic glaucoma	4
Neovascular glaucoma	5
Uveitic glaucoma	3
Previous penetrating keratoplasty and secondary glaucoma	1
Blunt trauma and angle recession	1
Long-term use of topical antiglaucoma medication	4

Method of application and surgical steps

Local anaesthetic in the form of a peribulbar block with 2.5 ml of 2% lignocaine was given. This was followed by cleaning of the operative field with 1% povidone iodine and sterile draping in all patients.

A limbus-based conjunctival flap followed by conventional trabeculectomy was performed in all ($n = 21$) eyes. During trabeculectomy, a cellulose sponge soaked in 0.2 mg/ml DNR was applied for 3 minutes at the proposed site of trabeculectomy before preparation of the partial thickness scleral flap. The sponge was removed after 3 min and the wound was irrigated with 15 ml Ringer lactate to wash off the unabsorbed drug. A two-third thickness limbus-based scleral flap of 5 mm width was made and an inner trabecular block of 1×2 mm size was excised. The superficial scleral flap was reposited and five sutures of 10-0 monofilament nylon were applied. Continuous suturing of conjunctiva was performed with 8-O vicryl. Postoperative, the patients received topical 0.1% betametasone q.d.s, 1% atropine sulphate b.d., and chloramphenicol eye drops q.d.s in the operated eye for 2 weeks. The surgical technique and postoperative care were essentially same in all patients.

Postoperative follow up

The postoperative observations were made every other day for the first week followed by 2–4, 5–8, 9–12, 13–24, 25–36, and 37–48 weeks. The follow-up was carried out for a minimum of 12 months in all cases.

Following parameters were observed at each study visit: VA, IOP, bleb characteristics, corneal change, fundus examination, and visual fields (where possible were carried out). Pre-and postoperative observations were made by the same examiner.

Criteria for success: IOP (measured by applanation tonometry) less than 21 mmHg without any antiglaucoma medication at 12-month review was taken as criteria for success.

Results were statistically analysed and the mean and standard deviation were calculated.

Results

Age and sex distribution

The mean age of patients at diagnoses was 27.91 ± 22.78 years (range 0.02–60 years).

Out of all the patients ($n=21$), there were 12 male patients and nine female patients in the study group.

Intraocular pressure

Following s/c DNR trabeculectomy, the IOP decreased from a basal preoperative mean value of 36 ± 5.9 to 5 ± 3.58 mmHg during the first postoperative week, 9.95 ± 3.70 mmHg at 2–4 weeks, 11.48 ± 2.96 mmHg at 5–8 weeks, and got stabilised by 9–12 weeks at 13.09 ± 2.83 mmHg. At the end of follow-up, that is 37–48 weeks the IOP was 16.05 ± 2.52 mmHg. Thus, following DNR trabeculectomy, the IOP was lowered from baseline preoperative mean value of 36.19 ± 5.9 mmHg on maximum tolerated antiglaucoma medication to 16.05 ± 2.52 mmHg on no antiglaucoma treatment at the end of 1 year. Success rate of 81% ($n=17/21$ patients) was noted at the end of 1-year follow-up (see Table 2).

Bleb characteristics

DNR blebs were diffuse, thick, and slightly pale (See Figure 1).

Visual acuity

Following DNR trabeculectomy, the VA was static in all 21 eyes at 1-year follow-up.

Complications

The most frequently observed complication in our study was shallow anterior chamber ($n=8/21$). The anterior chamber reformed spontaneously in all cases within first postoperative week. Complications such as wound leak, corneal epithelial toxicity, ocular hypotony, choroidal detachment, and hypotony maculopathy were not observed in any case.

Table 2 Decrease in IOP following DNR trabeculectomy

	Mean IOP ± range (mmHg)	*Fall in IOP from basal IOP (%)*
Basal (pre op)	36.19 ± 5.9	
Post op		
1st day	2.38 ± 1.24	93
3rd day	2.62 ± 1.60	93
5th day	3.62 ± 3.04	89
7th day	5 ± 3.58	85
2–4weeks	9.95 ± 3.70	70
5–8 weeks	11.48 ± 2.96	67
9–12 weeks	13.09 ± 2.83	63
13–24 weeks	14.48 ± 2.99	59
25–36 weeks	15.14 ± 3.13	57
37–48 weeks	16.05 ± 2.52	54

IOP, intra locular pressure; post op, post operative; pre op, pre operative.

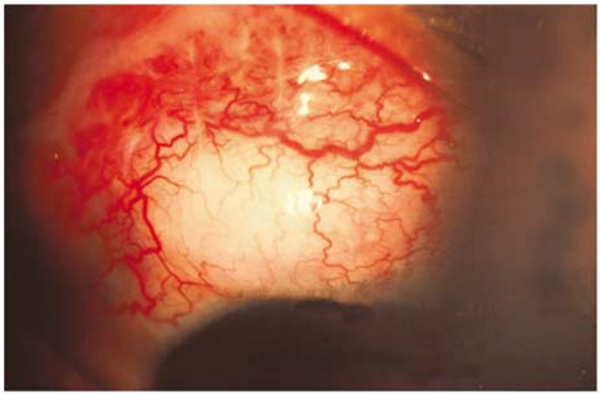

Figure 1 Filtering bleb: 3 months following daunorubicin-modulated trabeculectomy.

Discussion

The goal of a GFS is to control the IOP without undue side effects. GFS usually fails from postoperative fibroblast proliferation, collagen deposition, and subsequent sclerostomy or bleb scarring. High-risk factors for failure of GFS include young age, black race, aphakia, pseudophakia, trauma, previous ocular surgery, failed filtering surgery, developmental angle anomalies, phakomatoses, glaucoma secondary to neovascularisation or uveitis, and long-term use of antiglaucoma medication.[2–5] Pharmacological agents with antifibroblastic action have been used experimentally as well as clinically to modulate the process of wound healing and thus prevent scarring at the conjunctival–episcleral interface.[15] Antimetabolites such as MMC and 5-FU are the most commonly used agents as adjuncts in drug-modulated trabeculectomy.[16] Although these antimetabolites have been successful in enhancing IOP control, their use has been associated with side effects such as corneal erosions, leaking blebs, late endophthalmitis, cataract formation, and prolonged hypotony leading to maculopathy.[17,18] In this pilot study, an attempt has been made to evaluate the IOP lowering effect and safety of DNR, an antimetabolite with known antifibroblastic action.

Daunorubicin

DNR is an antitumor drug originally isolated in Italy as daunomycin[19] and independently in France as rubidomycin.[20] It is a fermentation product of the fungus *Streptomyces peucetius* vas caesius. DNR has a tetracycline ring structure with an unusual sugar, daunosamine, attached by glycosidic linkage. DNR is available as a

lyophilised powder in 20 mg vials. The dry, unopened vials are stable at room temperature; however, the drug should be used within 6 h after reconstitution with normal saline. Systemically it has been used in the treatment of non-lymphoblastic leukaemia. Systemic use of DNR is associated with bone marrow depression, gastrointestinal disturbances, and cardiac and dermatological side effects.[21] To the best of our knowledge, none of the workers have reported similar side effects with ocular use.

DNR has been seen to prevent proliferation of fibroblasts in experimental animals as well as in human eyes.[6] Although the antifibroblastic action of DNR has been known for over two decades, its safety in various ophthalmic surgeries has been demonstrated in the last decade. DNR has increasingly been used to improve the success rate of ocular surgeries, including strabismus surgery,[7] pterygium surgery,[8] retinal detachment surgery,[9] and in the prevention of PCO following cataract extraction.[10,11]

Dadeya *et al*[16,6] evaluated the safety and efficacy of intraoperative 0.02% DNR in strabismus surgery and pterygium surgery. In strabismus surgery, DNR was applied subconjunctivally over the surface of the extra ocular muscle and bare sclera for 3 min in patients undergoing second surgical procedure. They noted that a satisfactory alignment in primary position with improved ocular motility was obtained in DNR patients compared to the control group after 12 months of follow-up ($P = 0.16$). They recommended that intraoperative DNR is a useful adjunct in strabismus patients undergoing repeat surgery. In another study by the same workers, DNR was used in primary pterygium surgery in an attempt to decrease recurrence following excision. Intraoperative DNR was applied for 3 min during a bare sclera procedure. They reported that pterygium recurrence rates were less in patients treated with DNR and no postoperative complications were seen.

DNR has also been used in the treatment of experimental and clinical advanced proliferative vitreoretinopathy (PVR) after retinal detachment surgery.[9,21–24] Kumar *et al*[9] used DNR as an intravitreal injection in a dose of 5 μg during vitrectomy on patients' primary rhegmatogenous retinal detachment. The main outcome measures evaluated were retinal attachment, vitreous activity and VA at 3 months after surgery. They noted a statistically significant reduction in vitreous reaction with the use of DNR. Inoue *et al*[25] treated eyes with severe PVR undergoing vitrectomy with an infusion of DNR to prevent reproliferation. They concluded that DNR seemed to be effective to suppress reproliferation, but that care should be taken to avoid postoperative complications.[9,22–25]

Some workers[10,11] have performed research into use of DNR as an agent for preventing PCO. They noted that DNR could effectively inhibit the proliferation of human lens epithelial cells *in vitro* at the concentration of 0.5 μg/ml, almost reaching the greatest effect at the concentration of 7.5 μg/ml. They concluded that adjunctive use of DNR could reduce PCO formation by approximately 50%.

In glaucoma, the antifibroblastic action of DNR has been evaluated experimentally and clinically. Morales *et al*[15] used DNR implants (DNR conjugated to hyaluronic acid) in rabbits as an adjunct to full thickness sclerostomies. They concluded that DNR supplementation significantly improved the success rate of GFS. Rabowsky *et al*[12] studied the effect of a sustained subconjunctival release of DNR by way of a biodegradable polymer on the success of GFS in a rabbit model. IOP, bleb survival, and complications were evaluated. They reported that during the post operative period, decrease in IOP from baseline was significantly greater and bleb survival was significantly longer in the DNR-treated eyes than in the control group. There were no statistically significant differences between the DNR-treated and control eyes regarding corneal clouding, lens clarity, cataract formation, or conjunctival injection. Conjunctival erosions were noted in four DNR-treated eyes. Xu *et al*[13] used DNR as an adjunct to standardised partial thickness filtration surgery in rabbits. The animals received either 25 or 50 μg subconjunctival injections *vs* controls that received diluents. The filtration blebs lasted significantly longer ($P < 0.005$), the rate of fistula closure was significantly lower ($P < 0.005$), and the subepithelial connective tissue was much looser and thicker in the experimental eyes than in the control eyes. Corneal toxicity occurred more frequently in the eyes treated with the higher dose DNR than in those treated with the lower dose and in the control eyes.

Demailley *et al*[14] used DNR during trabeculectomy in human eyes. In a prospective randomised study, they compared the results at 20 months of preoperative S/C 5 FU *vs* S/C DNR injections before filtering surgery in two groups of 25 patients with primary OAG. The success rate was 79% with 5FU and 68% with DNR. They noted that complications such as flat anterior chamber, choroidal detachment, and corneal complications (corneal ulcer, corneal dystrophy) were less frequent in the DNR group. However, transient chemosis and local palpebral oedema were constant in DNR-treated eyes.

To the best of our knowledge, the intraoperative use of DNR in drug-modulated trabeculectomy has been demonstrated in our study for the first time.

The authors feel that intraoperative use of DNR during trabeculectomy improved the subconjunctival drainage of aqueous by inhibiting fibroblast proliferation at the site of filtration bleb, following subconjunctival

application at the site of trabeculectomy. The success rate of DNR trabeculectomy at the end of follow-up was 81%. Ophthalmic complications reported so far with DNR use include conjunctival dehiscence, scleral buckling, and orbital cellulites in patients receiving infusion for severe PVR.[26–28] In our study, no serious complications were observed. Except for the expected postoperative inflammation, we did not observe transient chemosis and local palpebral oedema or conjunctival erosions in any of our patients, as reported in previous studies.[14] A prospective case series comparing intraoperative DNR with intraoperative MMC during trabeculectomy has also been conducted by the authors. This is currently under review by an ophthalmic journal. DNR appears to show promise as an adjunct to GFS particularly in eyes with poor surgical prognosis for glaucoma.

Conclusion

DNR improved the subconjunctival drainage of aqueous by inhibiting fibroblast proliferation at the site of filtration bleb. The authors feel that intraoperative DNR is safe and effective in lowering IOP in high-risk surgical cases of glaucoma.

However, a much larger cohort study over a considerable number of years will eventually demonstrate its safety.

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