In Vitro Study of Photodynamic Therapy to control Postoperative Fibrosis after Trabeculectomy

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**Purpose:** To evaluate the tolerability and efficacy of carboxyfluorescein ester (BCECF - AM) as an adjunctive antifibrotic agent in a rabbit model of filtration surgery.

**Methods:** 20 pigmented rabbits 3-6 months old were used. It were divided into two groups, 10 rabbits each. Surgical procedure: sclerectomy & iridectomy were done for both eyes. Rt eye was operated after application of the photosensitizer as one single dose of BCECF-AM (80 μg). It was given subconjunctivally in the sclerectomy site 5.0 mm from the limbus 10 minutes before operation. Episclera and Tenons were irradiated for 8 minutes using the blue light, fixed at 2 cm from the target. Group I followed for 2 weeks and group II for 4 weeks. At the end of that period, rabbits were killed, enucleation was done and histopathological examination was performed.

**Results:** All eyes showed early hyperaemia of conjunctiva and hazy cornea. IOP was lowered and built up after 5 days in left eyes, while right eyes continued till 14 days, then built up gradually to preoperative level in the 3rd and 4th week (P< 0.001). Histopathology: the irradiated eyes showed minimal fibrosis, low vascularization of conjunctiva. At 4 weeks there was excess pigmentation of ciliary processes, destruction of periphery of the retina, thickening of choroid. Left eyes showed excessive fibrosis and vascularization of conjunctiva, no other changes.

**Conclusion:** PDT seems to be an antifibrotic agent which increase the efficacy and prolong the function of the filtering operation. Further studies are needed to standardize the different parameters like drug dose, light and irradiation area, further protection to the retina to improve the results and avoid the hazards.

Postoperative scarring of the filtering bleb is the most crucial factor in determining the short and long term outcome of modern glaucoma filtration surgery. Trabeculectomy is the preferred operation, retrospective study has shown a failure rate of up to 30% within 3 months after surgery.

Various methods have been used to avoid naturally occurring scarring of the filtering bleb, mostly the use of antimetabolites as 5-fluorouracil (5-FU) and Mitomycin C (MMC).\(^1\)

2,7-bis 2, carboxyethyl 5,6 carboxyfluorescein, acetoxyethyl ester (BCECF-AM) is an intracellularly acting photosensitiser. It is applied locally in its inactive form, which diffuses into adjacent cells, then it is cleaved and rendered fluorescent by intracellular esterases. Carboxyfluorescein is phototoxic for human Tenon fibroblasts.\(^2\)

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mmHg. It was dramatically reduced in all eyes within the second postoperative day. Left eyes (noninjected) in both groups recorded rise of IOP to preoperative values (18.29 ± 1.59 mmHg) within one week, and continued the same level till the end of the 2’d week in group I and till the 4th week in group II with no statistical significance (P > 0.05). Right eyes (injected) in both groups recorded reduced IOP over the 1st two weeks (12.56 ± 3.44 mmHg) with P < 0.05 statistically significant. In group II, IOP started to increase gradually till the end of the 4th week (17.42 ± 2.01 mmHg) with P > 0.05 statistically nonsignificant.

Wound healing could be clinically observed as closure of the filtration area by fibrosis. This process was more prominent in the noninjected left eyes which occurred at the end of the 1st week. On the other hand, the injected right eyes showed that 8 eyes had working sclerostomy site till the end of the 2nd week in group I, with statistical significance (P < 0.001). In group II there were working sclerostomy site in 7 eyes after 3 weeks with significant statistical difference (P < 0.001). At the end of the 4th week, all eyes showed closed sclerostomy site.

Histopathological study: figures 1-4.

Discussion

Antimetabolites as Mitomycin C (MMC) or 5-Fluorouracil (5-FU) are used perioperatively as antifibrotic agents. MMC is toxic to both vascular endothelial cells and fibroblasts. 5-FU is toxic to fibroblasts yet relatively harmless to vascular endothelial cells.³ Both agents can be delivered by soaking cellulose sponge with solutions of known concentration of these drugs, while the delivered amount of the drugs to the tissues is unknown. Although these agents have unquestionably improved filtering success rates in poor surgical
Photodynamic therapy (PDT) is a relative localized selective treatment. It requires a photosensitizer and exposure to a wavelength of light that the drug absorbs. The photophysical pathways produce reactive oxygen intermediates that may cause direct cytotoxicity or indirect effects by damaging the local vascular system. Hill et al 1997, studied Tin ethyl etipurpurin (SnET2) as a photosensitizer to investigate the feasibility of PDT as an alternative antifibrotic therapy for filtering surgery in a rabbit model. Their study showed long survival of the filtering bleb in two thirds of animals 4 weeks after treatment. Histopathology showed hypocellular conjunctival and subconjunctival tissues and closure of the sclerostomy by fibrovascular ingrowth, with small numbers of inflammatory cells. On the other hand, the control group demonstrated failed bleb within 10 days of surgery. Also surgery alone as well as surgery followed by 664-nm light irradiation lead to failure of blebs 7 days postoperatively.

In this work we tried to evaluate the feasibility of cellular fluorescence generated photoreaction products as a method to control postoperative fibrosis. The fluorescent probe; 2', 7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxyethyl ester (BCECF-AM) is a cell membrane permeable compound rendered membrane impermeable and fluorescent upon cleavage by intracellular esterases. Exposure of cells that have incorporated BCECF-AM to light at appropriate wavelength leads to cellular photoablation.

The clinical results showed that left eyes (noninjected) of groups I & II there was conjunctival
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Hyperaemia and corneal haze for short time nearly 5 days postoperatively. Intraocular pressure (IOP) was reduced in 1st two postoperative days and then raised up to the preoperative level in the 1st week and continued to the 2nd week in group I and up to the 4th week in group II. On the other hand, the injected right eyes showed that 8 eyes had working sclerostomy site till the end of the 2nd week in group I, while in group II there were working sclerostomy site in 7 eyes after 3 weeks. At the end of the 4th week, all eyes showed closed sclerostomy site.

The histopathology of left eyes showed conjunctival excess fibrosis and vascularization, the other tissues are within normal configuration in both groups I & II.

On the other hand, the right injected eyes showed conjunctival spacing, less fibrosis and less vascularization. Corneas and sclera were normal. The iris showed hemorrhage near the sclerostomy site. The periphery of the retina showed hyperpigmentation of RPE, degenerative changes of the photoreceptors and thickening of the choroid. Ciliary processes showed increased thickness of ciliary epithelium with excessive vascularization and haemorrhages.

Grisanti et al 1999 and 2000, in their pilot study found that in groups of rabbits which received; the photosensitizer without illumination, surgery without photosensitizer, exposure to illumination with blue light, and those who received 40 µg of BCECF-AM photoaablative therapy, seemed to be ineffective since surgical failure occurred such as in control group within 10 days. They used BCECF-AM at a concentration of 70-100 µg, where filtration surgery success could be prolonged for about 3 weeks. This was expressed both in lowered IOP levels and reduced fibrosis at the sclerostomy site. They concluded that both clinical and light microscopic examination revealed no detectable damage in adjacent non-illuminated tissues. However they proposed that ultrastructural changes should be evaluated in further studies. 9,2

Previous investigators studied the antifibrotic effect of a single dose of 80 µg of BCECF-AM during glaucoma filtration surgery in human eyes with a poor surgical prognosis. Their data revealed that success of trabeculectomy in human glaucomatous eyes with a poor surgical prognosis can be prolonged when cellular photoablation is applied during filtration surgery. After surgery with a mean follow up period of 38.1 days, mean IOP of 8 successful eyes was 16.6 mmHg (P < 0.001). One eye with pseudoexfoliation glaucoma had an IOP of 17 mmHg at the last examination but a topical antiglaucomatous medication (qualified success) was needed. Within the first 5 postoperative days, there was shallow AC, hypotony and choroidal detachment secondary to an IOP of less than 6 mm Hg in one eye that resolved without surgical intervention. None of the eyes demonstrated hypotonic maculopathy. No detectable tissue damage at the cornea or conjunctiva. No blebbits or endophthalmitis was observed in any eye. In two eyes; one with secondary glaucoma after trauma, and the other with Axenfeld-Rieger syndrome, both eyes showed scarring within 1 month after surgery despite the application of PDT.10,11

BCECF-AM is a lipophilic drug, it may diffuse into other tissues adjacent to the area of subconjunctival injection. In our study of PDT in normotensive eyes of rabbits, there was histopathological adverse effect on the periphery of the retina including the RPE and photoreceptors and the ciliary epithelium. This needs further study and evaluation.

Conclusion

Cellular photoablation seems to be an effective modality of treatment to control postoperative fibrosis in vitro and in vivo. But there are multiple factors as; the appropriate dose of the dye, the dose of light and its wavelength, the irradiation area and the mean time of exposure of the photosensitized cells to the light of the operating microscope. Multiple dosing may be altered in the future to improve the antifibrotic effect of photodynamic therapy during glaucoma surgery.

References


