·Basic Research·

Safety and efficacy of photodynamic therapy using BCECF-AM compared to mitomycin C in controlling post-operative fibrosis in a rabbit model of subscleral trabeculectomy

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Abstract

• AIM: To evaluate the safety and efficacy of cellular photoablation using BCECF-AM [2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester mixed isomers] as a method to control postoperative fibrosis in subscleral trabeculectomy (SST) compared to mitomycin C (MMC) in a rabbit model.

• METHODS: A comparative prospective case-control animal study was conducted. Fourteen rabbits were subjected to SST with intraoperative use of wound modulating agents (MMC or BCECF-AM) of the right eye (study groups I and II respectively) and SST without use of intraoperative wound modulating agents for the left eye (control group II). Two rabbits 4 eyes were considered as control group I with no surgical intervention. BCECF-AM was injected subconjunctivally 30min before surgery followed by intraoperative illumination with diffuse blue light for 10min. Antifibrotic efficacy was established by clinical response and histological examination. Clinical response was assessed by measuring intraocular pressure (IOP) at day 1, 3, 5, 7, 14, 21 postoperatively. Success was defined by >20.0% reduction in IOP from the preoperative values without anti-glaucoma medications.

• RESULTS: The mean percentage of reduction was 35.0% in the study group I with only one eye (14.3%) had 12.5% reduction. The mean percentage of reduction was 28.0% in the study group II with two eyes (28.6%) in study group II had 14.2% reduction each. Regarding the control group II, the mean percentage of reduction was 14.3% with 64.3% eyes had <20.0% reduction. There was a highly statistically significant difference between each of the study groups (right eyes) and the corresponding control group II (left eyes) as regards the mean postoperative IOP values started from day 5 in both study groups and this highly significant difference remained so till the end of the follow up period. Histologically, MMC treated blebs showed thinning of conjunctival epithelium with marked reduction of the goblet cells relative to control. Marked sub-epithelial edema was seen along with variable collagen dispersion. Mild cellularity was noted in sub-epithelial tissue. BCECF-AM treated blebs showed normal conjunctival epithelial thickness with abundant goblet cells. Mild sub-epithelial edema was noted along with moderate collagen dispersion. No histological abnormality was noted in the ciliary body or the cornea in any of the studied groups.

• CONCLUSION: Cellular photoablation using BCECF-AM is a safe and effective wound modulating agent to control postoperative fibrosis in trabeculectomy. However MMC considered as a more potent adjuvant to trabeculectomy than BCECF-AM in promoting IOP reduction.

• **KEYWORDS:** subscleral trabeculectomy; mitomycin C; photodynamic therapy; BCECF-AM; intraocular pressure; cellular photoablation
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INTRODUCTION

Subscleral trabeculectomy (SST) is the most frequently applied surgical method to reduce intraocular pressure (IOP) in patients with glaucoma. It is generally performed when medical therapy fails to adequately control IOP [1]. Excessive subconjunctival scarring following surgery is responsible for failure in the majority of cases [2-3]. There is a

huge interest in developing a new drug or treatment modality that would be able to minimize fibrosis and provide better outcome with surgery[4-10].

Antimetabolites, predominantly 5-fluorouracil (5-FU) and mitomycin-C (MMC) are commonly used to reduce the formation of scar tissue at the site of glaucoma filtering surgery [11]. These antimetabolites have been shown to be beneficial in preventing scarring and enhancing the long-term success, but they are relatively nonspecific and may be associated with an increased incidence of severe and potentially blinding complications[12-14].

Photodynamic therapy has also been evaluated for some distinct ophthalmological diseases such as ocular tumors, choroidal and corneal neovascularization, proliferative vitreoretinal disorders, and postoperative fibrosis in glaucoma surgery[15]. Photodynamic therapy might be an alternative way to solve this problem. Photosensitisers can be used as mediators of light induced cell toxicity. They seem to act *via* the formation of reactive oxygen intermediates and free radicals. Selective activation of the photosensitiser by light application at the appropriate wavelength limits the drug effect on the targeted area[16].

Several Photosensitisers are under investigation in ophthalmology. Cellular photoablation can be mediated by BCECF-AM [2', 7'-bis- (2-carboxyethyl)-5- (and-6)-carboxyfluorescein, acetoxymethyl ester mixed isomers] using a concentration of 70-100 μg [17]. BCECF-AM (carboxyfluorescein derivative) 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 the appropriate wavelength leads to cellular photoablation[18]. The light induced cytotoxic ability of BCECF-AM has been shown before *in vitro* studies. Human scleral and Tenon's capsule fibroblasts exposed to BCECF-AM for 45min at a concentration of approximately 9.2 μmol/L (corresponding to 0.4 μg) and irradiated by diffuse blue light for 1min resulted in 100% cell death. Cellular photoablation in contrast to chemotherapeutic agents acts only on the targeted cells[19].

The aim of this study was to evaluate the safety and efficacy of cellular photoablation using BCECF-AM as a method to control postoperative fibrosis in trabeculectomy compared to the effect of MMC combined with the same procedure in a rabbit model.

MATERIALS AND METHODS

A comparative prospective case-control animal study was conducted at Ophthalmology Department, Pathology Department and Medical Research Center, Faculty of Medicine, Ain Shams University in the period from March 2013 till August 2013. Thirty-two eyes of sixteen healthy adult New Zealand white male rabbits, weighing approximately 2.0-4.0 kg were locally bred at the animal house of Medical Research Center.

Study Design All experiment procedures conformed to the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research Involving Humans for studies involving experimental animals and human beings, respectively and the ARVO resolution on the use of animals in research and to institutional guidelines and performed according to the recommendations of Research Ethical Committee, Faculty of Medicine, Ain Shams University. Fourteen rabbits were subjected to SST with intra-operative use of wound modulating agents (MMC or BCECF-AM) of the right eye (study groups I and II respectively) and SST without use of intraoperative wound modulating agents for the left fellow eyes (control group II). The remaining two rabbits 4 eyes were considered as control group I.

The eyes were allocated into one of four groups: control group I: 4 eyes with no surgical intervention; control group II: 14 eyes had SST without intra-operative administration of wound modulating agents; study group I: 7 eyes had SST combined with intra-operative application of MMC (0.3 mg/mL); study group II: 7 eyes had SST combined with intra-operative application of BCECF-AM photosensitiser [dose of 80 μg in 300 μL balanced salt solution (BSS)].

All rabbits were examined preoperatively to exclude any ocular abnormalities. IOP was recorded using hand held Perkin's applanation tonometer (Haag-streit, USA). To exclude cyclic variations, IOP was compared between the right and left fellow eye preoperatively and postoperatively. The difference in measured IOP was expressed as right eye/left eye ratio.

Glaucoma Filtering Surgery Technique Each rabbit was anesthetized using a combination of ketamine injection (50 mg/kg; Egyptian International pharmaceutical industries company, Ketam, Egypt) in the auricular vein and intramuscular (xylazine 10 mg/kg). Additional topical anesthetic in the form of 0.4% benoxinate hydrochloride eye drops was administered.

In study group I, MMC (10 mg; Kyowa Biochem Pharmaceuticals' industries, India) was reconstituted by adding 15 mL of BSS to the vial. The vial was kept refrigerated. To withdrawn 1 mL from it and diluted with 1 mL of BSS to obtain a concentration of 0.3 mg/mL. A fornix-based conjunctival flap was prepared. A nearly half-thickness scleral flap, 3.0 mm ×4.0 mm, was dissected into clear cornea. In study group I, a cellulose microsponge soaked in 0.3 mg/mL MMC solution was applied under the conjunctiva over the scleral flap with the conjunctive draped over the sponge for 3min. The sponge was then removed and the entire area was lightly and copiously washed with irrigating saline. A standard trabeculectomy of equal size (1 mm×1 mm) was created, a peripheral iridectomy was made using scissors

and the scleral flap was repositioned without suturing. This was because of the aggressive wound healing response in rabbits which was equivalent to high risk eyes in humans and surgical failure result within one week [20]. So scleral suturing would aggravate the fibrotic response. Then the conjunctiva was closed using 10/0 interrupted nylon stitches.

In study group II, the samples of BCECF-AM for subconjunctival injection were prepared. Solid form 0.5 mg was provided by (Sigma-Aldrich, St Louis, MO, USA). A dose of 80 μg BCECF-AM was diluted in 300 μL BSS and stored at -20℃ as recommended by manufacturer. A single dose of BCECF-AM (80 μg in 300 μL BSS) was applied subconjunctivally in the region of the proposed filtering bleb before conjunctival incision and fashioning of the scleral flap. The injection was made 10 mm from the corneal limbus by a 27 gauge needle, a fornix-based conjunctival flap was performed, and the episcleral and subconjunctival Tenon's were irradiated for 10min, starting 15min after the injection, with blue light provided by direct ophthalmoscope (Heine Beta 200, Optotechnik, Germany) from a distance of approximately 10 cm. The choice of the dose and timing of application of BCECF-AM was according to Grisanti *et al*[17]. Subscleral trabeculectomy technique was used in control group II but without applying BCECF-AM or MMC. All surgeries were conducted by the same surgeon. Any intraoperative complication was recorded.

Postoperative Evaluation Tobradex eye drops (Tobramycin 0.3%, dexamethazone 0.1%, Alcon pharmaceutical, USA) was administered five times daily for three weeks. All rabbits were examined daily for evaluation of the bleb morphology and detection of any postoperative complications. IOP was measured at day 1, 3, 5, 7, 14, 21 postoperatively. Success was defined by ＞20.0% reduction in IOP from the preoperative values without anti-glaucoma medications. It also considered when IOP ratio between the right and left eye ＜0.85 which reflect the same success definition and this was determined prior to commencing the study.

Histopathology Animals were sacrificed after three weeks under general anesthesia. Eyes were enucleated, fixed immediately in formaldehyde 10% for at least 24h. The globes were then bisected vertically at the site of glaucoma surgery. The specimens were embedded in paraffin and sectioned with microtome. Sections (5-7 μm) were stained with hematoxylin-eosin for cellularity including fibroblast, Periodic acid-Schiff (PAS) for goblet cell identification, Masson's Trichrome (MT) for collagen density and architecture and Orcein stain for elastic fiber detection. The grading system to assess cellularity, collagen deposition and goblet cell number were calculated using the average cell number per high power field from central bleb cross-sections of each specimen. A masked evaluation was then performed on all samples.

Histological Morphometric Analysis Image analysis was performed using computerized Image Analyzing Software (Special SIS starter, version 3.2, Olympus, Germany) connected to an Olympus microscope (model BX51, Olympus, Japan) equipped with digital camera for histological grading. Cell counting was done in a masked fashion by one of the authors. Counts were recorded from six images of each conjunctival specimen (magnification ×400) from every group and the average number was taken in each group. Goblet cell density (number of cells/field) was graded as follows: 0.0: no goblet cells; +1.0: 1-20 cells; +2.0: 21-40 cells; +3.0: ＞40 cells. As regards cellularity of subepithelial and stromal tissues: 0.0 means absence of cells (inflammatory) per field; +1.0: 1-5 cells; +2.0: 6-10 cells. Absences of collagen fiber dispersion or condensation were graded as 0.0; +1.0 means 25% more dispersion or condensation as compared to control I group (mild); +2.0: 26%-50%(moderate);+3.0: >50% (severe). Presence of elastic fibers was graded as +1.0. The thickness of conjunctival epithelium and also the subconjunctival tissue in all groups was measured by the image analyzer and comparison was done with those of the control group I. Normal thickness was graded as 0.0 and thickened epithelium by 25% was graded as +1.0. 0.0: No subepithelial edema; +1.0: 25% increase in thickness of subepithelial tissue (mild edema); +2.0: 26%-50% (moderate edema), +3.0: >50% (severe edema).

Statistical Analysis All data were coded and statistically analyzed using the SPSS version 13.0 for windows (SPSS Inc., South Wacker Drive, Chicago, USA). Description of qualitative variables was in the form of numbers and percentages. Chi-square and Fisher's exact tests were used to compare the distribution of several histopathological features between the study groups. Student's t-test was used to compare quantitative data.The level $P \leqslant 0.05$ was considered the cut-off value for significance. Differences were considered highly significant when $P \leqslant 0.01$.

RESULTS

Among the operated eyes included in the study, no intraoperative complication was reported except one eye (3.6%) of total hyphema belonged to the study group I. It was completely resolved after 24h without surgical intervention. No post-operative complication during or at the end of follow up period was observed such as corneal erosions, flat anterior chamber, iris incarceration, blebitis, endophthalmitis or cystic blebs. Regarding the bleb morphology; in control group II, the blebs were small and vascularized (Figure 1A) however in the study group I, they were elevated and avascular (Figure 1B). In the study group II, blebs were elevated and less vascular than the control group II and remained so till the end of the three weeks (Figure 1C).

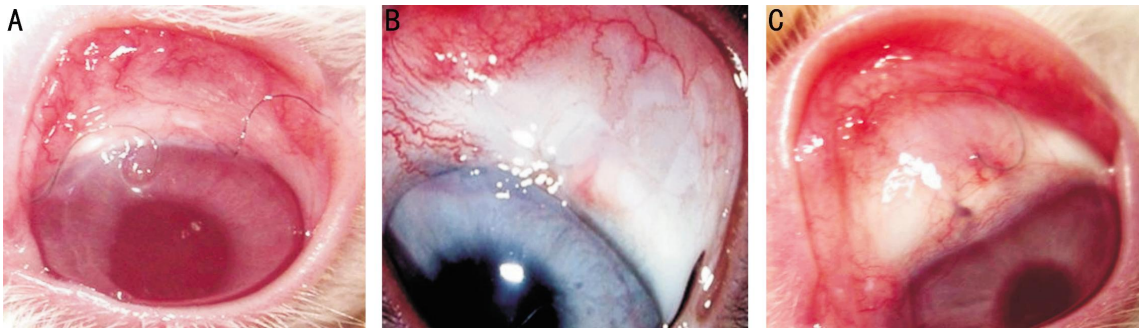

Figure 1 Bleb morphology at the end of follow up in the three groups A: In control group II (SST only), bleb is small and vascularized; B: In MMC treated group, it is elevated and avascular; C: In BCECF-AM treated group, it is elevated and less vascular than the control group II.

The mean preoperative IOP of the control group II was 7.8 (range: 7.0-9.0) mm Hg. In study group I and II it was 8.4 (range: 8.0-10.0) mm Hg and 7.8 (range: 7.0-9.0) mm Hg respectively. There was a reduction of the mean IOP postoperatively in both study groups compared to the mean preoperative values which was marked in the first day postoperatively. Gradual increase of the mean postoperative IOP was documented however at the end of three weeks, the mean percentage of reduction of IOP in the two study groups were ＞20.0% without anti-glaucoma medications. The mean percentage of reduction was 35.0% in the study group I with only one eye (14.3%) had 12.5% reduction (success rate 85.8%). The mean percentage of reduction was 28.0% in the study group II with two eyes (28.6%) in study group II had 14.2% reduction each (success rate 71.4%). Regarding the control group II, the mean percentage of reduction was 14.3% with 9 (64.3%) eyes had ＜20.0% reduction.

There was a highly statistically significant difference between each of the study groups (right eyes) and the corresponding control group II (left eyes) as regards the mean postoperative IOP values started from day 5 in both study groups and this highly significant difference remained so till the end of the follow up period (P-values were for study group I: 0.005, 0.0004, 0.000008 and 0.003 and for study group II: 0.007, 0.0003, 0.00005 and 0.008 for day 5, 7, 14 and 21 respectively). Figure 2 represented the mean preoperative and postoperative IOP values along the period of follow up in the study groups I and II compared to the control group II. The preoperative ratios (right/left) ranged from 0.87 to 1.28. The mean postoperative ratio reduced after surgery maximally within the second week of follow up and increased again at the end of follow up. The mean ratio at end of follow up was 0.79 (range: 0.7-1.0) in study group I and 0.84 (range: 0.6-1.0) in study group II. There was a highly statistically significant difference between the study group I and study group II as regards the mean right/left ratios started from day 5 postoperatively till the end of follow up periods (P-values were 0.004, 0.0005, 0.000001 and 0.0001 for days 5, 7, 14 and 21 respectively). Figure 3 demonstrated the pattern of these ratios along the period of follow up in both study groups.

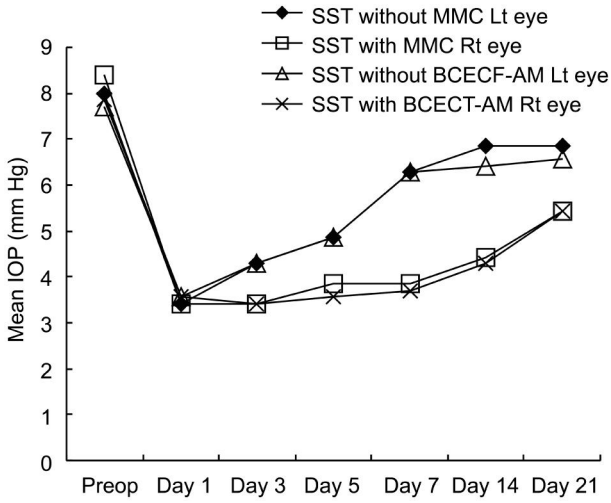

Figure 2 Comparison between the right eye (study group I: SST with MMC; study group II: SST with BCECF-AM) and the left eye (control group II, SST without MMC or BCECF-AM) as regards mean IOP (mm Hg) along the period of follow up.

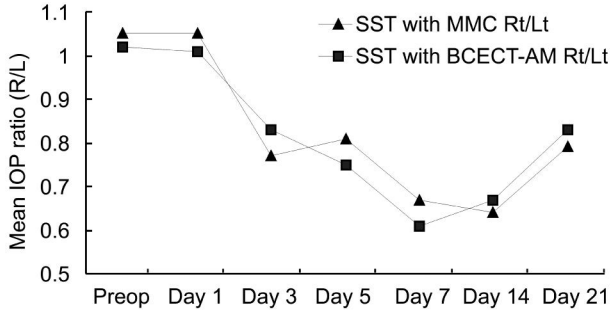

Figure 3 Mean IOP ratios between right (study groups) and left eyes (control group II) preoperatively and postoperatively along the period of follow up.

Histopathological Results Control group I showed normal epithelium with abundant goblet cells. Minimal to mild collagen dispersion was seen in the sub-epithelial connective tissue with scattered elastic fibers. There was no evidence of edema or interstitial cellularity (Figure 4).

Histological analysis of blebs of SST without wound modulating agents revealed normal conjunctival epithelial thickness with fewer goblet cells relative to control group. Sub-epithelial edema and collagen dispersion were graded as mild to moderate (Figure 5A and Figure 6A). Mild

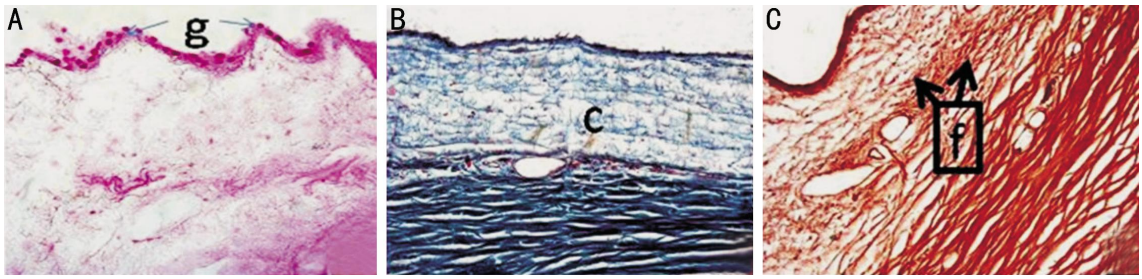

Figure 4 Conjunctiva of the normal control eyes A: Abundant goblet cells (g), normal epithelial thickness and architecture (PAS, ×200); B: Mild collagen (c) dispersion (MT, ×200); C: Scattered black elastic fibers (f) (Orcein, ×400).

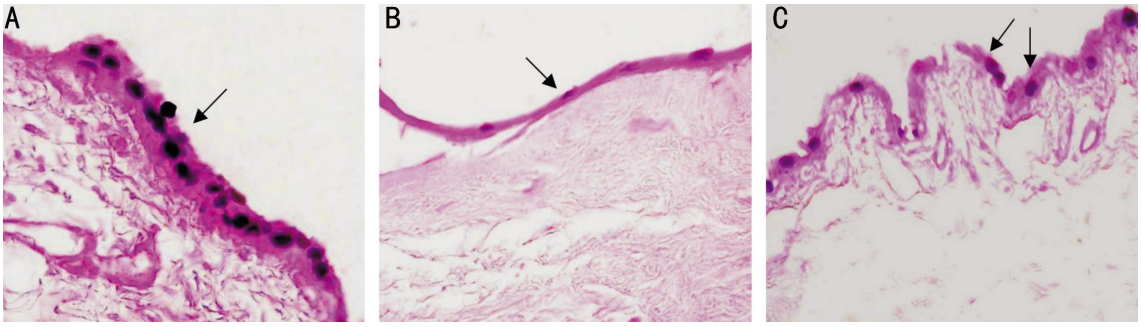

Figure 5 Conjunctiva of the control group II and study groups A: Normal epithelial thickness and number of conjunctival goblet cells (arrow) were noted in sections from SST blebs without antifibrotic; B: MMC treated tissue shows thinning of the conjunctival epithelium with loss of the normal distribution of the goblet cells (arrow) compared to the untreated eyes and BCECF-AM treated eyes; C: Normal conjunctival epithelial thickness and minimal reduction of the goblet cells (arrows) were noted in sections from BCECF-AM treated blebs (PAS, ×400).

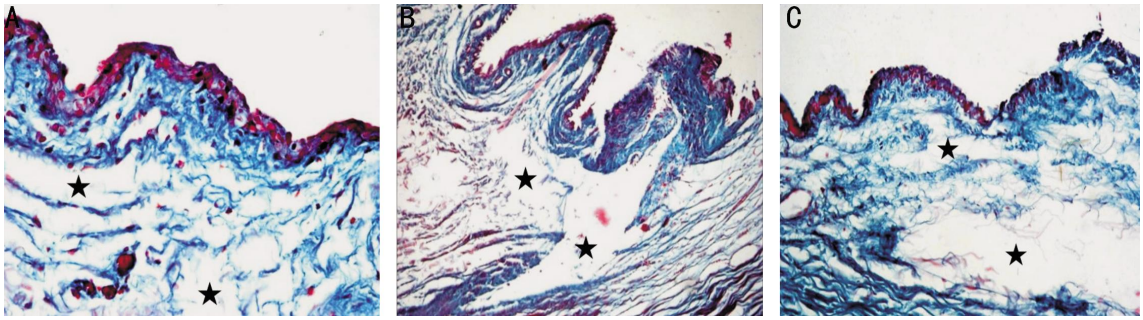

Figure 6 Collagen dispersion and sub –epithelial edema of bleb tissue A: Moderate collagen dispersion (asterisks) and moderate subepithelial edema in SST eyes not treated with antifibrotic agents as compared to the normal control group; B: MMC treated tissue shows marked collagen dispersion (asterisks) and marked subepithelial edema; C: Histological analysis of bleb tissue revealed less collagen deposition and moderate collagen dispersion (asterisks) and minimal sub-epithelial edema in BCECF-AM treated eyes compared to the control groups (MT, ×200).

sub-epithelial hypercellularity was noted in some cases. Scattered elastic fibers were also noted.

Tables 1 and 2 demonstrated comparison between the control and study groups as regards the presence of epithelial and stromal abnormalities and their statistical significance. Table 3 showed the histopathological difference between MMC and BCECF-AM treated blebs.

DISCUSSION

Unlike many other types of surgery in which complete healing of tissue with restoration of normal architecture would be a desirable outcome, glaucoma surgery seeks to achieve incomplete healing to allow aqueous humor to escape the eye. A completely healed trabeculectomy is a failed trabeculectomy. The use of antifibrotic agents to improve the success of glaucoma surgery has become common practice, and the benefits provided by these agents are accompanied by unique complications [21]. Especially for MMC, a high incidence of severe post application complications has been described, including thin and avascular filtering blebs, long lasting hypotony due to over filtration and ciliary body toxicity, and hypotony maculopathy with prolonged visual impairment, local inflammation, and even endophthalmitis [22]. In respect of 5-FU, in addition to its cytotoxic side effects mainly affecting the corneal epithelium, its clinical use is also limited by severe, applicational pain and discomfort for the patient[13]. To avoid toxic effects on intraocular tissues, it would be valuable to have an agent that will act only on the side of interest. This

Table 1 Comparison of the epithelium and stroma of the conjunctiva between eyes with no surgical intervention (control group I) and each of SST group without antifibrotic agents (control group II), SST with MMC (study group I) and SST with BCECF-AM (study group II)

Parameters	Control group I (4 eyes)		Control group II (14 eyes)		^1P	Study group I (7 eyes)		^2P	Study group II (7 eyes)		^3P
	Grade	*n* (%)	Grade	*n* (%)		Grade	*n* (%)		Grade	*n* (%)	
Normal epithelial thickness	0.0	2.0 (50.0)	0.0	13.0 (92.9)	0.81 (NS)	0.0	6.0 (85.7)	0.03 (SS)	0.0	7.0 (100.0)	0.06 (NS)
	+1.0	2.0 (50.0)	+1.0	1.0 (7.1)		+1.0	1.0 (14.3)		+1.0	0.0	
Goblet cell density	0.0	0.0	0.0	0.0	0.03 (SS)	+0.0	2.0 (28.6)	<0.01 (HS)	0.0	0.0	0.02 (SS)
	+1.0	0.0	+1.0	2.0 (14.3)		+1.0	5.0 (71.5)		+1.0	3.0 (42.9)	
	+2.0	3.0 (75.0)	+2.0	11.0 (78.6)		+2.0	0.0		+2.0	4.0 (57.1)	
	+3.0	1.0 (25.0)	+3.0	1.0 (7.1)		+3.0	0.0		+3.0	0.0	
Sub-epithelial edema	+1.0	4.0 (100.0)	+1.0	2.0 (14.3)	0.63 (NS)	+1.0	4.0 (57.1)	0.02 (SS)	+1.0	7.0 (100.0)	0.07 (NS)
	+2.0	0.0	+2.0	6.0 (42.9)		+2.0	2.0 (28.6)		+2.0	0.0	
	+3.0	0.0	+3.0	6.0 (42.9)		+3.0	1.0 (14.3)		+3.0	0.0	
Cellularity	0.0	3.0 (75.0)	0.0	0.0	0.005 (HS)	0.0	4.0 (57.1)	0.78 (NS)	0.0	6.0 (85.7)	0.35 (NS)
			+1.0	2.0 (14.3)		+1.0	3.0 (42.9)		+1.0	1.0 (14.3)	
	+2.0	1.0 (25.0)	+2.0	12.0 (85.7)							
Collagen dispersion	0.0	3.0 (75.0)	0.0	0.0	0.003 (HS)	0.0	0.0	0.002 (HS)	0.0	0.0	0.001 (HS)
	+1.0	1.0 (25.0)	+1.0	13.0 (92.9)		+1.0	1.0 (14.3)		+1.0	2.0 (28.6)	
	+2.0	0.0	+2.0	1.0 (7.1)		+2.0	4.0 (57.1)		+2.0	5.0 (71.5)	
	+3.0	0.0	+3.0	0.0		+3.0	2.0 (28.6)		0.0	0.0	
Collagen condensation	0.0	0.0	0.0	2.0 (14.3)	0.17 (NS)	0.0	7.0 (100.0)	< 0.01 (HS)	0.0	5.0 (71.5)	0.003 (HS)
	+1.0	4.0 (100.0)	+1.0	12.0 (85.7)		+1.0	0.0		+1.0	2.0 (28.6)	
Elastic fibers	+1.0	4.0 (100.0)	+1.0	14.0 (100.0)	(NS)	+1.0	7.0 (100.0)	(NS)	+1.0	7.0 (100.0)	(NS)

NS: No statistically significant; SS: Statistically significant; HS: Highly statistically significant. ^1P: Control group II *vs* control group I; ^2P: Study group I *vs* control group I; ^3P: Study group II *vs* control group I.

Table 2 Comparison of the epithelium and stroma of the conjunctiva between SST group without antifibrotic agents (control group II) and each of SST with MMC (Study group I) and SST with BCECF-AM (study group II)

Parameters	Control group II (14 eyes)		Study group I (7 eyes)		^1P	Study group II (7 eyes)		^2P
	Grade	*n* (%)	Grade	*n* (%)		Grade	*n* (%)	
Normal epithelial thickness	0.0	13.0 (92.9)	0.0	6.0 (85.7)	0.49 (NS)	0.0	7.0 (100.0)	1.0 (NS)
	+1.0	1.0 (7.1)	+1.0	1.0 (14.3)		+1.0	0.0	
Goblet cell density	0.0	0.0	+0.0	2.0 (28.6)	< 0.01 (HS)	0.0	0.0	0.019 (SS)
	+1.0	2.0 (14.3)	+1.0	5.0 (71.5)		+1.0	3.0 (42.9)	
	+2.0	11.0 (78.6)	+2.0	0.0		+2.0	4.0 (57.1)	
	+3.0	1.0 (7.1)	+3.0	0.0		+3.0	0.0	
Sub-epithelial edema	+1.0	2 (14.3)	+1.0	4.0 (57.1)	0.59 (NS)	+1.0	7.0 (100.0)	1.0 (NS)
	+2.0	6 (42.9)	+2.0	2.0 (28.6)				
	+3.0	6 (42.9)	+3.0	1.0 (14.3)				
Cellularity	0.0	0.0	0.0	4.0 (57.1)	< 0.01 (HS)	0.0	6.0 (85.7)	< 0.01 (HS)
	+1.0	2.0 (14.3)	+1.0	3.0 (42.9)		+1.0	1.0 (14.3)	
	+2.0	12.0 (85.7)						
Collagen dispersion	+1.0	13.0 (92.9)	+1.0	1.0 (14.3)	<0.01 (HS)	+1.0	2.0 (28.6)	<0.01 (HS)
	+2.0	1.0 (7.1)	+2.0	4.0 (57.1)		+2.0	5.0 (71.5)	
	+3.0	0.0	+3.0	2.0 (28.6)				
Collagen condensation	0.0	2.0 (14.3)	0.0	7.0 (100.0)	<0.01 (HS)	0.0	5.0 (71.5)	0.001 (HS)
	+1.0	12.0 (85.7)	+1.0	0.0		+1.0	2.0 (28.6)	
Elastic fibers	+1.0	14.0 (100.0)	+1.0	7.0 (100.0)	(NS)	+1.0	7.0 (100.0)	(NS)

NS: No statistically significant; HS: Highly statistically significant; SS: Statistically significant. ^1P: Study group I *vs* control group II; ^2P: Study group II *vs* control group II.

problem might be solved by cellular photoablation using light-absorbing chemicals[23].

BCECF-AM is an intracellularly acting photosensitiser. It is applied locally in its inactive form, diffuses into adjacent cells, and is then cleaved and rendered fluorescent by intracellular esterases [13]. After additional illumination (activation) with blue light, it exerts a photo oxidative effect that is only cell destructive within the targeted cells[24]. Hill

Table 3 Comparison of the epithelium and stroma of the conjunctiva between SST group with MMC (Study group I) and SST group with BCECF–AM (study group II)

Parameters	Study group I (7 eyes)		Study group II (7 eyes)		*P*
	Grade	*n* (%)	Grade	*n* (%)	
Normal epithelial thickness	0.0	6.0 (85.7)	0.0	7.0 (100.0)	0.001 (HS)
	+1.0	1.0 (14.3)	+1.0	0.0	
Goblet cell density	+0.0	2.0 (28.6)	0.0	0.0	< 0.01 (HS)
	+1.0	5.0 (71.5)	+1.0	3.0 (42.9)	
	+2.0	0.0	+2.0	4.0 (57.1)	
	+3.0	0.0	+3.0	0.0	
Sub-epithelial edema	+1.0	4.0 (57.1)	+1.0	7.0 (100.0)	0.003 (HS)
	+2.0	2.0 (28.6)			
	+3.0	1.0 (14.3)			
Cellularity	0.0	4.0 (57.1)	0.0	6.0 (85.7)	0.04 (SS)
	+1.0	3.0 (42.9)	+1.0	1.0 (14.3)	
Collagen dispersion	+1.0	1.0 (14.3)	+1.0	2.0 (28.6)	0.008 (HS)
	+2.0	4.0 (57.1)	+2.0	5.0 (71.5)	
	+3.0	2.0 (28.6)	+3.0	0.0	
Collagen condensation	0.0	7.0 (100.0)	0.0	5.0 (71.5)	0.04 (SS)
	+1.0	0.0	+1.0	2.0 (28.6)	
Elastic fibers	+1.0	7.0 (100.0)	+1.0	7.0 (100.0)	(NS)

HS: Highly statistically significant; SS: Statistically significant; NS: No statistically significant. *P*: Study group I *vs* study group II.

et al [16] investigated the feasibility of photodynamic therapy in a rabbit model of filtration surgery. Using ethyl etiopurpurin, a photosensitiser traditionally delivered by intravenous injection, they showed that after subconjunctival injections, large areas of avascular conjunctiva were produced and filtering bleb survival was prolonged.

The aim of this study was to evaluate photodynamic therapy for antifibrosis using BCECF-AM. Exposure of cells that have incorporated BCECF-AM to light at the appropriate wavelength leads to cellular photoablation. The light induced cytotoxic ability of BCECF-AM had confirmed before in multiple *in vitro* studies with cochlear outer hair cells and dermal fibroblasts[25].

Further, this effect is strictly limited to the local restriction of the illuminated area. *In vitro*, carboxyfluorescein was shown to be phototoxic for human Tenon's fibroblasts which were the major cells types involved in subconjunctival fibrosis and bleb failure after filtration surgery [23]. *In vivo*, in a rabbit model of filtration surgery, Grisanti *et al* [17] investigated the impact of cellular photoablation mediated by BCECF-AM on fibrosis after surgery. They used different concentrations of the photosensitizer with or without intraoperative illumination with diffuse blue light or complete illumination. They did not use MMC in their study. They found that using BCECF-AM at a concentration of 70-100 μg filtration surgery success could be prolonged for about 3wk. This was expressed both in lowered IOP levels and reduced fibrosis at the sclerostomy site.

In the present study, BCECF-AM was tested in a rabbit model of filtration surgery. The efficacy of the photodynamic effect was clinically represented by a functioning filtering bleb with a reduced IOP level which was maximum in the first week maintained till end of second week and start to rise again by third week and these results were comparable to results of MMC group.

MMC treated blebs clinically appeared more avascular and histologically showed significant thinning and alteration of the conjunctival tissue morphology in the form of marked collagen dispersion and this was comparable to the results published by Sherwood[26]. The marked reduction in collagen deposition responsible for that MMC group achieved the highest percentage of success (85.7%) and the lowest percentage of failure over the follow-up period. The use of MMC may be associated with an increased incidence of adverse effects, including hypotony-maculopathy, bleb leaks, bleb infections, and endophthalmitis [27], however this not encountered in our study. Trabeculectomy alone resulted in the highest percentage of failure (64.3%) in our study. The difference between the groups as regards IOP reduction was marked, it was a highly statistically significant ($P<0.01$). Thus, combining trabeculectomy with adjunctive BCECF-AM or MMC makes the procedure more efficient.

The clinical safety and tolerability of photodynamic therapy was represented by no signs of local toxicity or intraocular inflammation, and the lack of any adverse. No severe complications were seen in any of the eyes included in this study.

Though the applied carboxyfluorescein, as a lipophilic drug, could easily penetrate into adjacent superficial ocular tissues, no conjunctival or corneal-epithelial defect was observed in

any eye postoperatively. As outlined above, the dye is applied preoperatively, subconjunctivally, and the tissue is irradiated before preparing the artificial fistula. Therefore, it is unlikely for carboxyfluorescein to penetrate into the eye. As a consequence and as already proved by histological analysis of rabbit eyes treated with carboxyfluorescein, ciliary body toxicity was excluded. However, ultrastructure should be evaluated in further studies.

The absence of any inflammatory response may be explained by Grisanti *et al* [23] study that involving carboxyfluorescein photoablation of human scleral and Tenon's fibroblasts in culture. They noticed that the photoablative effect did not lead to an acute release and accumulation of cellular debris. The affected cells were obviously damaged as shown by the cell viability tests but were not disrupted. Similarly targeted cells in the wound-healing model were affected and remained inert at the wound edge, but were not disrupted causing cellular debris. This should have a beneficial effect by avoiding the recruitment of inflammatory cells by necrotic tissue. Theoretically, cells from regions which were not targeted by photoablation may participate at the scarring process through proliferation and migration. However, it was assumed that the wound healing process will quiet down before cells from distant regions will reach the area of interest.

Grisanti *et al* [17] evaluated the effect of daylight on scleral fibroblasts that had incorporated BCECF-AM and could exclude a lethal effect. This result might be because of the lesser light intensity than that from a microscope. Furthermore, in the animal model and clinical practice, the upper lid will protect the injected area. A more intense light exposure will occur during surgery because of the operating microscope. They found that it was ineffective, and surgical failure occurred and concluded that when one of the components (photosensitiser, illumination with blue light) necessary for photoablation is missing, wound closure occurs and IOP will increase within 1wk.

Our findings support the utility of BCECF-AM for wound modulation in a rabbit model of filtration surgery. The results of this study documented that use of BCECF-AM resulted in improved bleb morphology and histology compared to the MMC group. In the current study, BCECF-AM treated eyes demonstrated favorable bleb morphology and histology. The stable density of goblet cells combined with modest fibroblast proliferation and collagen deposition suggest that the use of BCECF-AM may allow for the formation of thicker, more stable blebs. Use of BCECF-AM could allow for combination therapy with MMC or 5- FU at lower doses with decreasing the incidence of MMC related complications. A multicenter randomized clinical study involving large number of cases conducted over a long period of time is required to clarify long term safety and efficacy of the drug.

In conclusion, cellular photoablation using BCECF-AM is a safe and effective wound modulating agent to control postoperative fibrosis in trabeculectomy. However MMC considered as a more potent adjuvant to trabeculectomy than BCECF-AM in promoting IOP reduction.

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REFERENCES

1 Shigeeda T, Tomidokoro A, Araie M, Koseki N, Yamamoto S. Long-term follow-up of visual field progression after trabeculectomy in progressive normal-tension glaucoma. *Ophthalmology* 2002; 109(4):766 -770.

2 Seibold LK, Sherwood MB, Kahook MY. Wound modulation after filtration surgery. *Surv Ophthalmol* 2012;57(6):530-550.

3 Holló G. Wound healing and glaucoma surgery: modulating the scarring process with conventional antimetabolites and new molecules. *Dev Ophthalmol* 2012;50:79-89.

4 Wang F, Qi LX, Su Y, Yan QH, Teng Y. Inhibition of cell proliferation of Tenon's capsule fibroblast by S-phase kinase-interacting protein 2 targeting SiRNA through increasing p27 protein level. *Invest Ophthalmol Vis Sci* 2010;51(3):1475-1482.

5 O'Neill EC, Qin Q, Van Bergen NJ, Connell PP, Vasudevan S, Coote MA, Trounce IA, Wong TT, Crowston JG. Antifibrotic activity of bevacizumab on human Tenon's fibroblasts in vitro. *Invest Ophthalmol Vis Sci* 2010;51(12): 6524-6532.

6 Horsley MB, Kahook MY. Anti-VEGF therapy for glaucoma. *Curr Opin Ophthalmol* 2010;21(2):112-117.

7 Duffield JS, Lupher ML Jr. PRM-151 (recombinant human serum amyloid P/pentraxin 2) for the treatment of fibrosis. *Drug News Perspect* 2010;23(5):305-315.

8 Lukowski ZL, Min J, Beattie AR, Meyers CA, Levine MA, Stoller G, Schultz GS, Samuelson DA, Sherwood MB. Prevention of ocular scarring after glaucoma filtering surgery using the monoclonal antibody LT1009 (Sonepcizumab) in a rabbit model. *J Glaucoma* 2013;22(2):145-151.

9 Du LQ, Yang HL, Wu XY, Wang SG, Li Y. Effect of poly (DL-lactide-co-glycolide) on scar formation after glaucoma filtration surgery. *Chin Med J (Engl)* 2013;126(23):4528-4535.

10 Van Bergen T, Jonckx B, Hollanders K, Sijnave D, Van de Velde S, Vandewalle E, Moons L, Stassen JM, Stalmans I. Inhibition of placental growth factor improves surgical outcome of glaucoma surgery. *J Cell Mol Med* 2013;17(12):1632-1643.

11 Yoon PS, Singh K. Update on antifibrotic use in glaucoma surgery, including use in trabeculectomy and glaucoma drainage implants and combined cataract and glaucoma surgery. *Curr Opin Ophthalmol* 2004;15 (2):141-146.

12 Jampel HD, Quigley HA, Kerrigan-Baumrind LA, Melia BM, Friedman D, Barron Y; Glaucoma Surgical Outcomes Study Group. Risk factors for late-onset infection following glaucoma filtration surgery. *Arch Ophthalmol* 2001;119(7):1001-1008.

13 Simsek T, Firat P, Citirik M, Ozdamar Y, Elgin U. Short-term effects of subconjunctival injections of 5-fluorouracil on conjunctival epithelium. *Cornea* 2010;29(7):727-731.

14 Green E, Wilkins M, Bunce C, Wormald R. 5-Fluorouracil for glaucoma surgery. *Cochrane Database Syst Rev* 2014;2:CD001132.

15 Smyth RJ, Nguyen K, Ahn SS, Panek WC, Lee DA. The effects of photofrin on human Tenon's capsule fibroblasts *in vitro*. *J Ocular Pharmacol* 1993;9(2):171-178.

16 Hill RA, Crean DH, Doiron DR, McDonald TJ, Liaw LH, Ghosheh F, Hamilton A, Berns MW. Photodynamic therapy for antifibrosis in a rabbit model of filtration surgery. *Ophthalmic Surg Lasers* 1997;28(7):574-581.

17 Grisanti S, Diestelhorst M, Heimann K, Krieglstein G. Cellular photoablation to control postoperative fibrosis in a rabbit model of filtration surgery. *Br J Ophthalmol* 1999;83(12):1353-1359.

18 Jordan JF, Diestelhorst M, Grisanti S, Krieglstein GK. Photodynamic modulation of wound healing in glaucoma filtration surgery. *Br J Ophthalmol* 2003;87(7):870-875.

19 Dougherty TJ. An update on photodynamic therapy applications. *J Clin Laser Med Surg* 2002;20(1):3-7.

20 Khaw PT, Doyle JW, Sherwood MB, Smith MF, McGorray S. Effects of intraoperative 5-fluorouracil or mitomycin C on glaucoma filtration surgery in the rabbit. *Ophthalmology* 1993;100(3):367-372.

21 Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. *Surv Ophthalmol* 2003;48(3):314-346.

22 DeBry PW, Perkins TW, Heatley G, Kaufman P, Brumback LC. Incidence of late-onset bleb-related complications following trabeculectomy with mitomycin. *Arch Ophthalmol* 2002;120(3):297-300.

23 Grisanti S, Gralla A, Maurer P, Diestelhorst M, Krieglstein G, Heimann K. Cellular photoablation to control postoperative fibrosis in filtration surgery: in vitro studies. *Exp Eye Res* 2000;70(2):145-152.

24 Grimes PA, Stone RA, Laties AM, Li W. Carboxyfluorescein. A probe of the blood-ocular barriers with lower membrane permeability than fluorescein. *Arch Ophthalmol* 1982;100(4):635-639.

25 Dulon D, Zajic G, Schacht J. Photo-induced irreversible shortening and swelling of isolated cochlear outer hair cells. *Int J Radiat Biol* 1989;55(6):1007-1014.

26 Sherwood MB. A sequential, multiple-treatment, targeted approach to reduce wound healing and failure of glaucoma filtration surgery in a rabbit model (an American Ophthalmological Society Thesis). *Trans Am Ophthalmol Soc* 2006;104:478-492.

27 Mac I, Soltau JB. Glaucoma-filtering bleb infections. *Curr Opin Ophthalmol* 2003;14(2):91-94.