EXTENDED REPORT

Photodynamic modulation of wound healing in glaucoma filtration surgery

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Aim: To report a clinical pilot study investigating photodynamic therapy (PDT) in combination with glaucoma filtration surgery. BCECF-AM was used as the photosensitising substance. The clinical safety and tolerability of BCECF-AM, and its efficacy in controlling postoperative intraocular pressure (IOP) were assessed.

Methods: Before trabeculectomy (TE), 42 consecutive eyes of 36 glaucoma patients received one subconjunctival injection of 80 µg BCECF-AM (2,7,-bis- (2-carboxyethyl) -5- (and-6) -carboxy-fluorescein, acetoxymethyl-ester) followed by an intraoperative illumination with blue light (λ = 450–490 nm) for 8 minutes. Antifibrotic efficacy was established as postoperative IOP reduction of >20% and/or an IOP constantly < 21 mm Hg without antiglaucomatous medication. Follow up of the filtering bleb was documented by slit lamp examination.

Results: Eyes had mean 1.1 preoperative surgical interventions (filtration and non-filtration glaucoma surgery). Mean preoperative IOP was 31.6 (SD 9.7) mm Hg. Patients were followed for mean 496 days (range 3.5–31.8 months). Of the 42 eyes, 25 eyes had an IOP decreased to 15.8 (3.4) mm Hg without medication (complete success: 59.5%; p<0.001; *t* test). Seven eyes showed good IOP reduction < 21 mm Hg under topical antiglaucomatous medication (qualified success: 16.7%). 10 eyes failed because of scarring within 2–67 weeks (23.8%). Clinical follow up examinations revealed no local toxicity, no uveitis, and no endophthalmitis.

Conclusions: This method is a new approach in modulating postoperative wound healing in human eyes undergoing glaucoma filtration surgery. The data of the first human eyes combining TE with PDT underline the clinical safety of this method and its possible potential to prolong bleb survival.

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 (TE) is the preferred operation. However, in conventionally performed trabeculectomy, a large retrospective study has shown a failure rate of up to 30% within 3 months after surgery.[1]

To lower the incidence of this detrimental complication, various methods have been investigated in order to avoid the naturally occurring scarring of the filtering bleb, mostly dealing with the intraoperative or postoperative application of antimetabolic drugs—that is, 5-fluorouracil (5-FU) or mitomycin C (MMC), the two most widely used cytotoxic agents.[2 3]

BCECF-AM (2,7,-bis-(2-carboxyethyl) -5- (and -6) -carboxyfluorescein, acetoxymethyl-ester) 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.[4–6] After additional illumination (activation) with blue light, it exerts a photo-oxidative effect that is only cell destructive within the targeted cells.[7–10] Further, this effect is strictly limited to the local restriction of the illuminated area.[11]

In vitro, carboxyfluorescein was shown to be phototoxic for human Tenon fibroblasts.[11] In vivo, in a rabbit model of filtration surgery, its potential to significantly delay postoperative scarring has also been demonstrated.[12] A first clinical pilot study comprising 10 eyes with end stage glaucoma and poor clinical prognosis has underlined the impact of cellular photoablation on postoperative fibrosis in glaucoma patients undergoing trabeculectomy, mediated by BCECF-AM based photodynamic therapy.[13]

This open label phase II study was performed to further investigate the clinical tolerability, safety and efficacy of trabeculectomy combined with BCECF-AM based photodynamic therapy.

METHODS

Patients

In all, 42 consecutive glaucomatous eyes of 36 patients were included in this study (surgery and clinical follow up). Before surgery, all eyes showed progressive, glaucomatous optic disc cupping and visual field impairment. In all eyes, intraocular pressure (IOP) was not controlled despite maximum tolerable topical and systemic antiglaucomatous therapy.

The study was approved by the ethics review board of the University of Cologne. It followed the guidelines of the declaration of Helsinki as revised in Tokyo and Venice. All patients signed an informed consent and were explained in detail about the purpose of the study and method of surgery.

Photosensitiser, photoactivation, and photo-oxidative effect

The fluorescent probe BCECF-AM (2,7,-bis- (2-carboxyethyl) -5- (and-6) -carboxy-fluorescein, acetoxymethyl-ester) was used in this study as the photosensitising agent. BCECF-AM, in its applied form, is a membrane permeable compound. Intracellularly, BCECF-AM is activated upon cleavage by intracellular esterases, thereby rendered fluorescent and membrane impermeable.[4–6] After additional illumination with diffuse blue light at the appropriate wavelength (λ = 450–490 nm, intensity ~51.9 × 1000 cd/m^2), intracellular carboxyfluorescein then exerts the photo-oxidative effect by producing free oxygen radicals (cytodestructive oxidative stress, photo-oxidative reactions of type I and II), finally leading to the death of the cell.[7–10]

Table 1 Demographic and clinical data of the patients' eyes treated with combined trabeculectomy and photodynamic therapy, using BCECF-AM as the photosensitising agent

Patient No	Age	Sex	Diagnosis	SH	Preop IOP	Preop Meds	OP	Postop IOP	LSL	Postop compl	FU (days)	FU-IOP	Red from preop IOP	Postop Meds
1	87	M	SEC		40	SCI; BB	1/99	13			405	18	–55%	
2	73	F	PEX		20	SCI; TCI; PGA	2/99	2			31	18		TCI; BB; PGA
3	20	M	AR	TE, 2×CC, CP	30	SCI; BB; PGA	1/99	16			14	scar		
4	37	F	SEC	TO, CC, 4×CP	45	SCI; TCI; AA	1/99	14			14	scar		
5	43	M	SEC	TE	24	AA; BB; M; PGA	1/99	5		H	937	18	–25%	
6	84	M	SEC	TEM	41	SCI; BB; M; PGA	3/99	7		H	953	7	–83%	
7	82	F	POAG	CP	31	BB	12/99	14			60	20		BB
8	60	M	POAG		50	SCI; BB; M	3/99	9			473	scar		
9	81	F	PEX	2×TE	40	SCI; BB; PGA	4/99	17			335	19		BB; PGA
10	82	F	PEX	2×TE, CP	50	TCI; PGA	4/99	3		B	63	scar		
11	31	M	POAG		35	SCI; PGA	11/99	10			144	19	–46%	
12	84	F	POAG		28	SCI; AA; PGA	11/99	10	×1		459	20	–29%	
13	69	F	ACG	TE	20	SCI; PGA	10/99	8			852	12	–40%	
14	60	F	DPOAG	LTP, DS	25	M; TCI; BB; PGA	12/99	10	×1		829	14	–44%	
15	24	F	DPOAG	TE	38	SCI	10/99	1		H+Healon	850	16	–58%	
16	21	F	CG	3×CC	38	SCI; AA; PGA	10/99	8			56	scar		
17	78	F	PEX		18	TCI; BB	7/00	6			365	14		TCI
18	76	F	ACG		26	SCI; AA; PGA	1/00	12	×1		670	18	–31%	
19	61	F	POAG	2×CP, TE	50	AA; PGA	5/00	2			469	19	–62%	
20	20	F	DPOAG	3×TE, TEM	52	SCI	11/00	2			21	scar		
21	63	F	POAG		24	BB; PGA	3/00	2			90	13		BB
22	43	F	SEC	3×TE, TEM	26	SCI; AA; PGA	1/00	6			14	scar		
23	53	F	POAG	1×LTP	36	SCI; AA; PGA	5/00	14	×1		656	20		BB
24	53	F	POAG		30	SCI; AA; PGA	7/00	10	×2		608	20		BB
25	45	F	POAG		48	SCI; BB; AA	5/00	1			612	17	–65%	
26	54	F	PEX		35	SCI; M	3/00	1			725	19	–46%	
27	82	F	POAG	TE	25	TCI; AA; PGA	10/00	12	×1		504	20	–20%	
28	82	F	POAG	TE	32	TCI; AA; PGA	1/01	4	×1		172	scar		
29	74	F	POAG	TE, LTP	27	AA; BB	9/00	8	×1		485	18	–33%	
30	31	F	SEC		42	SCI	1/01	30			30	scar		
31	60	F	DPOAG		20	TCI; BB; PGA	1/01	7			423	18	–10%	
32	64	M	POAG		27	SCI	1/01	12	×1		225	18	–33%	
33	36	M	SEC	TEM	31	SCI; TCI; AA	12/00	7			308	scar		
34	58	F	POAG	TE, CP	19	TCI; AA; PGA	1/01	18			413	12	–37%	
35	58	F	POAG	CP	30	AA; PGA	3/01	3			365	12	–60%	
36	60	M	POAG	TE	24	AA; PGA	5/01	12			134	12	–50%	
37	66	M	POAG		26	TCI; BB; AA	1/01	5	×1		159	14	–46%	
38	73	M	POAG		30	TCI; AA; PGA	5/01	10			261	13	–57%	
39	74	M	POAG		18	M; AA; PGA	2/01	5	×1		517	15	–17%	
40	74	M	POAG		28	M; AA; PGA	2/01	12	×1		498	14	–50%	
41	38	M	PDG		28	M; BB; PGA	5/01	4			407	17	–39%	
42	75	M	POAG		21	TCI	10/01	17			104	16	–24%	

Abbreviations; AA = α2 agonist; ACG = angle closure glaucoma; AR = Axenfeld-Rieger-syndrome; B = hyphaema; BB = β blocker; CC = cyclocryocoagulation; CG = congenital glaucoma; CP = cyclophotocoagulation; DPOAG = dysgenetic primary open angle glaucoma; FU = follow up; FU-IOP = intraocular pressure at the latest follow up; H = hypotony; LSL = laser suture lysis; LTP = (argon) laser trabeculoplasty; IOP = intraocular pressure; M = miotic; Meds = medications; OP = date of operation (month/year); PEX = pseudoexfoliation glaucoma; PGA = prostaglandin analogue; PIGG = pigment dispersion glaucoma; POAG = primary open angle glaucoma; postop Compl = postoperative complications; preop IOP = preoperative intraocular pressure; Red from preop IOP = percentage of reduction from preop IOP to latest postop IOP; scar = failure due to scarring; SCI = systemic carboanhydrase inhibitor; SEC = secondary glaucoma; SH = surgical history; TCI = topical carboanhydrase inhibitor; TE = trabeculectomy; TEM = trabeculectomy combined with mitomycin C.

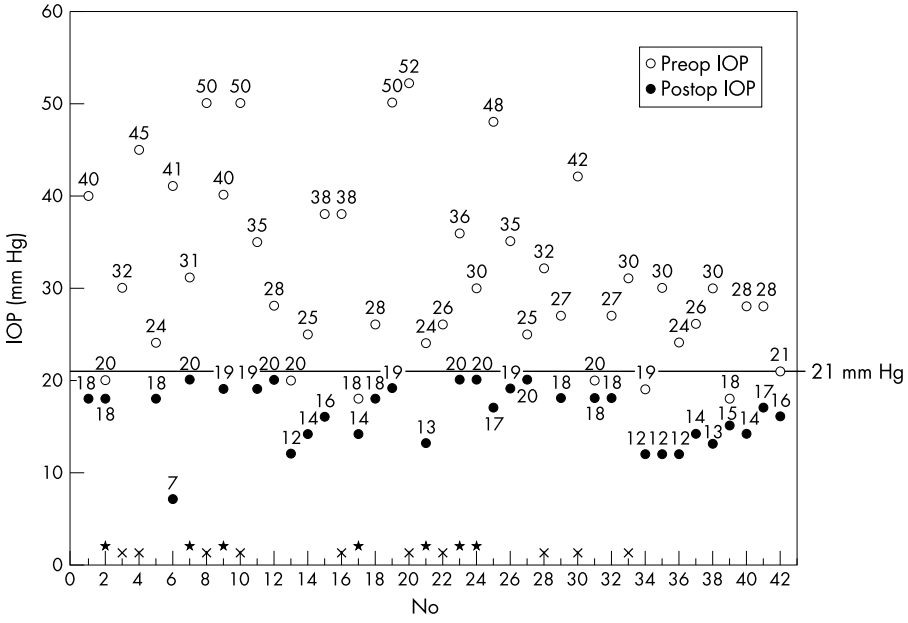

Figure 1 Preoperative and postoperative intraocular pressure (IOP) of each eye. IOP for each eye in mm Hg. For the eyes that had failed due to scarring (marked by X), no postoperative IOP is displayed. *Marks eyes resembling qualified success.

The applied samples of the photosensitiser were provided by the pharmacy of the University of Cologne in tuberculin syringes. A dose of 80 µg BCECF-AM was diluted in 300 µl of balanced salt solution (BSS) and stored at −80°C.

Surgical procedure and clinical follow up

Patients were hospitalised before surgery and remained in hospital until the fifth postoperative day. For the clinical follow up, patients were examined for the safety, tolerability and efficacy of the surgical procedure (described in detail below) 2 weeks and 1, 3, 6, and 12 months after surgery, respectively.

The surgical procedure was performed under general or topical anaesthesia by one surgeon (MD). One single dose of BCECF-AM (80 µg in 300 µl BSS) was applied by subconjunctival injection in the region of the later filtering bleb, 15 minutes before surgery. The injection was made in a 10 mm distance from the corneal limbus. A 27 gauge needle was used.

Trabeculectomy (TE) with photodynamic therapy (PDT) was performed as follows. Following a limbus based conjunctival flap, the episcleral and the adjacent subconjunctival Tenon were irradiated for 8 minutes with blue light at the appropriate wavelength, focused on the area where the trabeculectomy was to be performed.

Photoactivating light was provided by a portable lamp with flexible fibre optics, equipped with a special blue filter (Zeiss, Germany). The lamp was fixed at a distance of approximately 2 cm above the episclera. The cone of light was about 15 mm in diameter, illuminating the episcleral and subconjunctival tissue. The cornea was covered by a tape. Since the illuminated area was well focused, no further mask was needed to minimise light dispersion to adjacent tissues.

Within the illuminated area, consequently a scleral flap of 3.5 mm × 3.5 mm was prepared, followed by a standard trabeculectomy and a full thickness iridectomy. The scleral flap was closed with two 10/0 Nylon sutures. Tenon capsule and conjunctiva were closed with one 8/0 Vicryl running suture. At the end of the surgical procedure, all eyes were given a subconjunctival injection of water based dexamethasone and gentamicin solution.

After surgery, prednisolone acetate and tobramycin eye drops were administered for 14 days. No antimetabolites were applied, and no topical or systemic antiglaucomatous medication was given. The postoperative protocol only allowed subconjunctival injection of a water based bethamethasone solution, and the laser suture lysis of the two scleral 10/0 Nylon sutures.

Efficacy, defined as postoperative IOP reduction, was established by measuring the IOP by Goldmann applanation tonometry.

Complete success was defined as an IOP reduction of >20% and/or an IOP constantly <21 mm Hg without the need for antiglaucomatous medication.

Qualified success was defined as an IOP < 21 mm Hg under topical antiglaucomatous medication.

Eyes with an IOP >21 mm Hg despite topical antiglaucomatous therapy or the need for further surgical interventions were classified as failure or scarred, respectively.

Follow up examinations included visual acuity testing, thorough slit lamp examination, and photodocumentation of the anterior segment and the filtering bleb to assess the tolerability and safety of BCECF-AM injections. The photographs were taken with a 35 mm camera system (Nikon).

Statistics

A two tailed Student's *t* test was performed to assess the significance of postoperative IOP reduction in the complete success group. Statistical significance was assumed if $p < 0.05$.

RESULTS

In all, 42 consecutive eyes of 13 male and 23 female patients were included in this study. Mean age of the patients was 58, with a range of 20–87 years. All eyes had mean 1.1 preoperative surgical interventions (filtration and non-filtration glaucoma surgery). The preoperative intraocular pressure (IOP) in the 42 eyes ranged from 18 mm Hg to 52 mm Hg under maximum systemic and topical antiglaucomatous therapy, with a mean of 31.6 (SD 9.7) mm Hg. Patients received mean 2.5 antiglaucomatous medications preoperatively. See Table 1 for the detailed, patient related data.

Assessment of efficacy

The IOP during the first postoperative days ranged from 1 mm Hg to 30 mm Hg, with a mean of 8.8 (5.9) mm Hg. After a mean follow up of 496 days (range 104–953 days), out of the 42 eyes, 25 eyes showed an IOP reduction of >20% from preoperative IOP level and/or an IOP constantly reduced below 21 mm Hg, to mean 15.8 (3.4) mm Hg (59.5%, complete success). Seven eyes showed IOP reduction below 21 mm Hg under topical antiglaucomatous therapy to mean 17.7 (3.0)

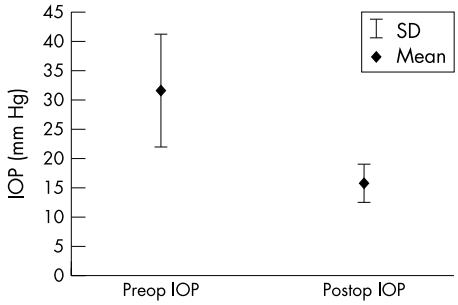

Figure 2 Statistical analysis of preoperative and postoperative intraocular pressure of the eyes resembling complete success (n=25). Mean (SD) preoperative and mean postoperative intraocular pressure (IOP) of the eyes with complete success is displayed. The result proved to be significant by p<0.000 (two tailed Student's *t* test).

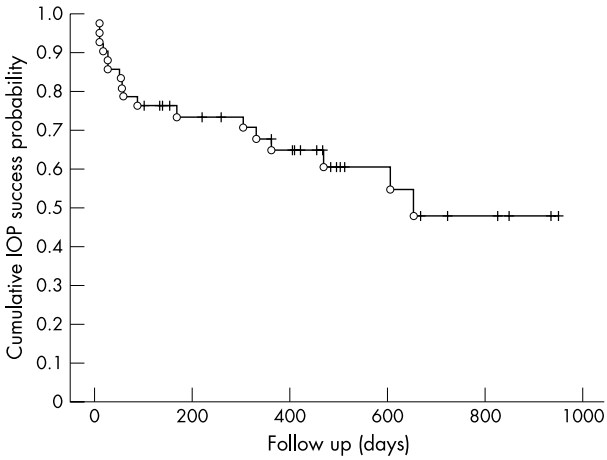

Figure 3 Kaplan-Meier analysis of cumulative IOP success: complete success of combined trabeculectomy and photodynamic therapy over time. End of follow up of the eyes to be classified as "qualified success" or "failure due to scarring" is marked by a circle.

mm Hg (16.7%, qualified success). Ten eyes failed due to scarring after 14–469 days (2–67 weeks) postoperatively (23.8%), resembling a pronounced wound healing process despite the application of BCECF-AM and photodynamic therapy.

Figure 1 gives an overview of the preoperative and postoperative IOP for each eye.

The postoperative reduction in IOP in the eyes resembling complete success proved to be significant by p <0.000 in the two tailed Student's *t* test (Fig 2).

See Figure 3 for the Kaplan-Meier cumulative survival analysis. Complete success of combined trabeculectomy and photodynamic therapy is displayed over time.

Assessment of clinical safety and tolerability

Within the first 5 days postoperatively, only mild conjunctival hyperaemia and mild anterior chamber flare was detected in most patients. The patients did not report of pain or uncomfortable sensations, unusual or severe postoperative symptoms, or complications.

One patient showed a mild postoperative hyphaema resolving spontaneously. Three patients developed a shallow anterior chamber with mild choroidal detachment due to postoperative hypotony (IOP <6 mm Hg), one needing a Healon injection, otherwise resolving spontaneously and without the need for further surgical intervention. No hypotony maculopathy was seen in these eyes.

With respect to the application of BCECF-AM, no tissue damage of the conjunctiva or the cornea was seen. No inflammation of the bleb or its surrounding tissue was observed. There were no signs of endophthalmitis in any of the eyes.

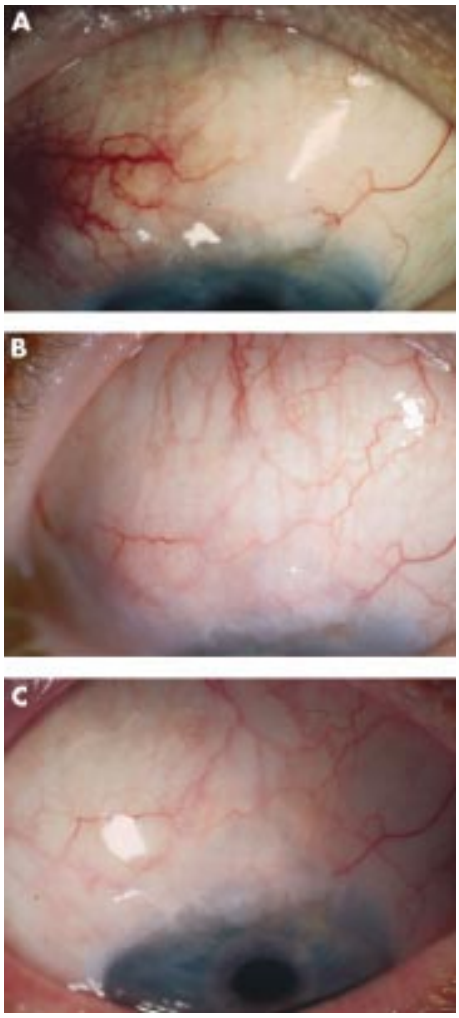

Figure 4 Photographs of a representative filtering bleb during the 1 month (A), 9 month (B) and 13 month (C) clinical follow up examination after combined trabeculectomy and photodynamic therapy with BCECF-AM. Note that there are no signs of avascularity, cystic encapsulations, inflammation, or local toxicity.

Postoperative bleb appearance was variable, but remained within the usual postoperative limits. Avascularity of the filtering bleb, as it is known to appear after the application of antimetabolites, was not seen.

See Figure 4 for the representative course of a filtering bleb after TE + PDT, at the 1 month, 9 months, and 13 months postoperative follow up examination.

DISCUSSION

The complex issue of modulating the wound healing process after glaucoma filtration surgery has been the target of many experimental, as well as clinical studies.[2] Until today, no sufficient method has been established to achieve satisfying postoperative long term surgical results with only minimal or no side effects for the patient.

The antimetabolites mitomycin C (MMC) and 5-fluorouracil (5-FU) are most widely used, both intraoperatively and postoperatively, to suppress scarring of the filtering bleb which would mean failure of the filtration surgery.[3 14–18] Despite their positive long term effect on prolonged filtration, the application of cytotoxic drugs to a surgically opened eye increases the incidence of severe, iatrogenic complications.

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 overfiltration and ciliary body toxicity (as MMC easily diffuses into the eye), hypotony maculopathy with prolonged visual impairment, local inflammation, and even endophthalmitis.[3 15 19–23] 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.

Photodynamic therapy in ophthalmological diseases has been used in humans for the treatment of ocular tumours,[24 25] proliferative vitreoretinal disorders, choroidal and corneal neovascularisation,[26–29] and in a rabbit model of filtration surgery,[30] using different photosensitising agents with different specific properties.[31 32]

In this study, we investigated a new method combining the advantages of photodynamic therapy and trabeculectomy. BCECF-AM was used as the photosensitising agent. The photoablative potential of BCECF-AM has been described previously,[10 33] and was confirmed for human Tenon fibroblasts before this study.[11 12]

In our study comprising 42 human glaucoma eyes, the efficacy of the photodynamic effect was clinically represented by a functioning filtering bleb with a reduced IOP level. The clinical safety and tolerability was represented by no signs of local toxicity or intraocular inflammation, and the lack of any discomfort or adverse effects for the patient.

No severe complications as avascularity of the filtering bleb, long lasting hypotony, hypotony maculopathy, blebitis, uveitis, phthisis, or endophthalmitis 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, evaluated by thorough slit lamp examination 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 analyses of rabbit eyes treated with carboxyfluorescein,[12] ciliary body toxicity can be excluded.

After a mean follow up of 496 days, out of the 42 eyes, successful filtration could be observed in 25 eyes (59.5%), represented by an IOP of mean 15.8 mm Hg in glaucomatous eyes with a mean 1.1 presurgical interventions (filtration and non-filtration glaucoma surgery). Seven eyes (16.7%) showed good IOP control under topical antiglaucomatous medication. Ten eyes (23.8%) failed due to scarring. These 10 eyes had mean 2.5 presurgical interventions, and included four uveitic secondary glaucomas (Nos 4, 22, 30, 33). Though it has not been further investigated yet, this probably reflects that under the above regimen of intraoperative BCECF-AM based TE + PDT, the application of mitomycin C or 5-fluorouracil in eyes with highly "boosted" scar forming tissue might still be superior to photodynamic therapy so far.[34]

In the Fluorouracil Filtering Surgery Study, the success rate is reported to be 73% after 1 year and 51% after 3 years for the 5-FU group. For the standard treatment group, a failure rate of 50% after the first year and 74% after the 3 year follow up period (or success of 50% and 26%, respectively) is reported.[35 36] In a very recent study on the TGF-β antibody CAT-152 as adjuvant in combination with trabeculectomy, the reported success rate was 69% after one year in the CAT-152 group. For the placebo group, they report a success rate of 25% that could only be increased by the additional application of 5-FU.[37]

The efficacy of BCECF-AM based photodynamic therapy compared to standard trabeculectomy has to be validated in randomised and placebo controlled clinical studies. An appropriate multicentre trial is approved.

Cellular photoablation using BCECF-AM seems to be a feasible new method in combination with glaucoma filtration surgery. This is underlined by the data of the first 42 human eyes combining TE and PDT. Parameters such as light dose, irradiation area, time of application and irradiation, total dosage of the dye, or even multiple dosing may be altered in the future to improve the antifibrotic effect of BCECF-AM.

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