Effects of mitomycin C in early conjunctival inflammation after pterygium surgery.

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Abstract

Purpose

The purpose of this study was to compare inflammatory events and graft characteristics one and 6 months after conjunctival limbal autograft (CLAU) with and without intraoperative mitomycin C (MMC).

Methods

This study included 69 eyes of 69 patient's eyes with pterygium. Clinical data concerning patient demography; preoperative examination including pterygium morphology, recurrence clinical assessment, and complications after CLAU with (MMC+) and without (MMC-) intraoperative MMC were all registered at 1 and 6 months after surgery.

Results

Thirty-five eyes were included in MMC+ and 34 in MMC-. Preoperative data were similar in both groups (Student t test and Fisher's exact test;; $p<0.05$).

Thirty four (49.6 %) eyes in the whole sample showed at least one inflammatory complication at 1 month after surgery. MMC- group showed a significantly higher number of cases with complications ($p <0.001$; Chi2 test). (MMC+ 28.5%; MMC-70.5%).No patients presented clinical recurrence at 1 month after surgery. The examination revealed a higher incidence of clinical hyperemia surrounding the surgical site and graft contraction on the host site on the MMC- group, both with statistical signification ($p <0.001$; Chi2 test). Although the hemorrhages were less frequent in the MMC– group there were no significant differences between the two groups ($p >0.05$; Chi2 test). Pyogenic granuloma developed at the surgical site in 3 eyes (4.37%), 2 of those granulomas were at the MMC– group ($p>0.05$).

Tendency for recurrences was significantly different between both groups ($p=0.0001$; Fisher's exact test) at the end of 6 months. Thirteen (38%) eyes showed recurrence in MMC- and no cases were displayed in MMC+. Presence

of at least 1 inflammatory event was only seen in 16 (23%) cases, all of them in MMC-. Specifically, 15 (44%) eyes showed hyperemia and one (3%) eye presented conjunctival hemorrhages. No new cases of pyogenic granuloma or graft contraction were seen at this time point in both groups. Hyperemia was the only specific event with significant differences between MMC- and MMC+ (p= 0.0001; Fisher's exact test) at 6 months after surgery.

Conclusion

The eyes receiving intraoperative MMC after CLAU seems to present less hyperemia and graft contraction after surgery than those that did not receive MMC as an adjuvant factor. Intraoperative MMC could be associated with a lower recurrence rates.

Introduction

Pterygium is a pathological progressive growth of fibrovascular conjunctival tissue from bulbar conjunctiva towards the center of the cornea. Although initially, it was described as a chronic degenerative disease[1], histopathological findings like proliferative cells in its head and the presence of different inflammatory markers found in its tissue[2] have recently changed this concept towards the proliferative and inflammatory nature of the lesion.[2]

Many adjuvant treatments associated with pterygium surgery have been established over the past years in order to reduce recurrences, like the application of cytostatics such as mitomycin C (MMC)[3] and 5-fluorouracil[4], the application of amniotic membrane[5,6] and conjunctival or conjunctival limbal autograft (CLAU) placement[7]. Recently, it has been demonstrated that adjuvant combination in the same surgery procedure yields a lower recurrence rate. Indeed, the combination of conjunctival or limbal autograft with MMC reduces more the recurrence rate than the same treatment without MMC application.[8] Chan et al[9], recently demonstrated that conjunctival autograft (CAU) and intraoperative MMC are a safe option when used together in pterygium surgery.

Although in this study, CAU seems to have lower long-term recurrence rate compared to MMC over bare sclera in double-head pterygium surgery.

MMC is an alkylating antitumor antibiotic isolated from *Streptomyces caespitosu*. It inhibits the fibrovascular activity in the pterygium stroma by inhibiting DNA synthesis[10]. This prevents aggressive wound healing, reducing pterygium recurrence after bare sclera excision[5,11,12]. Studies in rabbits revealed that after repeated subconjunctival injections of 0.1ml of MMC, the fibroblast and vessels disappeared from the conjunctiva and Tenon capsule, and collagen fibers decreased with time[12]. It has been suggested that MMC can reduce inflammation but it has not yet been demonstrated in human studies[5].

Untreated persistent inflammation may lead to a poor surgical outcome[6]. Moreover, it has been demonstrated that the treatment of this inflammation improves the final postoperative outcome[5].

The aim of this study was to compare inflammatory events and graft characteristics between those patients that underwent CLAU with and without intraoperative MMC at 1 month and 6 months after surgery.

Materials and Methods

Human subjects

This is a prospective, interventional study that included 69 eyes of 69 patients with pterygium and was conducted at Terrassa Health Consortium, from January 2013 to July 2015. It is a randomized controlled study with masked observers.

Patients with a history of contact lens wear, previous surgery, temporal or double-head pterygium, symblepharon, and other conjunctival lesion than pterygium were excluded.

The study was approved by the Terrassa Health Consortium Ethics Committee and informed consents were obtained from patients. The research adhered to the tenets of the Declaration of Helsinki.

Clinical data concerning patient demography; preoperative examination including clinical morphology of the lesion (the grade of visibility of episcleral vessels) were assessed under biomicroscopy by a single masked observer.Thus, pterygia were classified as atrophic when episcleral vessels were clearly distinguished, fleshy when they were totally obscured, and intermediate when the vessels were distinguished with difficulty as previously described[7]. Postoperative inflammatory events and clinical adverse effect of the drug were registered (see below for details). Recurrence assessment, using Prabhasawat criteria[13], was also carried out 1 month and 6 months after lesion excision.

Procedure

All surgeries were performed by the same surgeon using bare sclera excision under topical anesthesia. The dissection of Tenon's during the surgery was as minimal as possible taking into account the extent of the pterygium. MMC 0.025% was applied intraoperatively (in 35 eyes out of the 69 eyes) (MMC+) in a soaked cellulose sponge to the scleral bed for 5 min followed by a CLAU, attached with a interrupted 10 nylon suture with no scleral bites in both groups (MMC+; MMC-). After surgery, a bandage contact lens was applied until corneal reepithelization was completed and all patients received an identical regimen of topical chloramphenicol and dexamethasone eye drops (De Icol, Alcon, BCN-Spain) 4 times a day for 1 week and tapered off in 1 month's time. Sutures were removed one week after surgery.

Postoperative Follow-Up

Comprehensive slit-lamp examinations of the anterior segment were taken at 1 month and 6 months after surgery in order to determine the presence of inflammatory events such as hyperemia, hemorrhage, graft contraction and pyogenic granuloma.

The hyperemia of the graft's surrounding conjunctiva was graded using the clinical classification previously described by Kheirkhah[7] (absence: 0; mild: 1; moderate: 2 and severe: 3) (Figure 1). Presence of graft contraction was graded

1 and established when graft limits were clearly distinguished and showed some degree of dehiscence at 4 weeks after surgery. Absence of graft contraction was graded 0 and implied a uniform conjunctiva with normal appearance. (Figure 2)

Presence or absence of hemorrhage and pyogenic granuloma (absence: 0; presence: 1) were also recorded.

Data Processing

Chi2 test was used to assess differences about number of events between groups (MMC+ vs MMC-). Fisher's exact test was used to separately analyze differences in each inflammatory event.

Results

Sixty-nine eye of 69 patients (43 (62.3%) males and 26 (37.7%) females) completed the follow up period. Mean age of the patients was 42.4 years (range 24 to 79 years). Sixty two (90%) eyes had primary pterygium and 7 (10%) eyes had recurrent pterygium.

Thirty-five eyes underwent through CLAU with mitomycin C (group MMC+) and thirty-four eyes CLAU without mitomycin C (group MMC-). There were no significant differences in age (p=0.268; student t test), sex (p=0.687; Chi2 test), race, lesion morphology, lesion area, grade of preoperative hyperemia and grade of visibility of episcleral vessels between the groups (Table 1). Surgery was uneventful, and secure attachment of the CLAU was obtained in all cases at the end of the surgical procedure.

Inflammatory events at 1 month after pterygium excision

No clinical drug adverse effects or recurrence cases were recorded at 1 month after surgery. Distribution of the number of inflammatory events in each group at 1 month after surgery is presented in Table 2. Thirty four (50 %) eyes in the whole sample showed at least one complication and MMC- group showed

significantly higher number of cases with complications (p <0.001; Chi2 test) (Table 2).

The examination revealed clinical significant conjunctival hyperemia surrounding the surgical site in 31 (45%) eyes of the whole sample and this inflammatory event was significantly lower in the MMC + group (p <0.001; Chi2 test) (Table 2).

Graft contraction on the host site was found in 21 of 69 eyes (30%). This clinical feature showed a significantly higher incidence in the MMC- group (p <0.001; Chi2 test) (Table 2).

The persistence of hemorrhages one month postoperatively was registered in 11 of 69 eyes (16%). Although these hemorrhages were less frequent in the MMC+, there were no significant differences between the two groups (p>0.05; Chi2 test). Pyogenic granuloma developed at the surgical site in 3 eyes (4 %), 2 of those granulomas were at the MMC– group (p>0.05). It is worth mentioning that the 3 cases which initially presented granuloma resolved with the sub-conjunctival injection of triamcinolone.

Inflammatory event at 6 months after pterygium excision

Tendency for recurrences was significantly different between both groups (p=0.0001; Fisher's exact test) at the end of the follow-up period. Thirteen (38%) eyes showed recurrence (grade 4 of Prabhasawat criteria12) in MMC- and no cases were displayed in MMC+.

Presence of at least 1 inflammatory event was only seen in 16 (23%) cases, all of them in MMC-. Specifically, 15 (44%) eyes showed hyperemia and one (3%) eye presented conjunctival hemorrhages. No new cases of pyogenic granuloma or graft contraction were seen at this time point in both groups. Statistical analysis showed significant tendency to higher number of inflammatory events in MMC- (p=0.0001; Fisher's exact test). Presence of conjunctival hyperemia

was the only specific event with significant differences between MMC- and MMC+ (p= 0.0001; Fisher's exact test) at 6 months after surgery.

Discussion

The presence of certain degree of conjunctival inflammation is usually regarded as a normal wound healing process after many conjunctival procedures[14-17]. However, it has been suggested that the persistence of postoperative inflammation might lead to recurrences and unsatisfactory aesthetic outcomes[6]. Factors that may play a role in this postoperative inflammation are mostly unknown.

The results of our study assessed the early effect of the use of intraoperative MMC on the postoperative conjunctival inflammation after the CLAU surgery. In the present study, the conjunctiva was examined in a follow-up period of 1 month and 6 months in order to determine the postoperative inflammatory degree of each group. It is accepted that postoperative inflammatory responses could subside one month after the surgery and many studies[5,6] have used this time point to compare the effect of different surgical techniques on the conjunctival condition.

Because pterygium morphologic features could affect the surgical outcome[5], eyes were previously evaluated preoperatively in order to dismiss any condition that could have influenced the results. No significant differences in type, clinical morphology and preoperative hyperemia of the pterygium were found between the groups. In addition, no patients presented clinical recurrence at 1 month after surgery.

Adjuvant techniques like intraoperative MMC, 5 FU, conjunctival autograft, amniotic membrane transplantation[18], subconjunctival MMC injection[11], and subconjunctival Ranibizumab have been widely evaluated to assess their role in preventing recurrence[19], but few studies have been focused on postoperative inflammation.

Keirkhah and associates[6] noted that Fibrin Glue produced less postoperative inflammation than sutures in those patients with conjunctival autograft surgery

(61.5% vs 21.4%, respectively). Later they found less inflammation on those eyes treated with conjunctival autograft than the ones treated with amniotic membrane transplantation (15 % vs 84.2%, respectively). The results of our study complement these findings showing that the use of intraoperative CLAU with MMC seems to improve the conjunctival condition after the surgery decreasing some inflammatory events. As a consequence of this, we might conclude that the effect of the MMC could increase the healing of the CLAU. Further assessments with a larger sample size are necessary to confirm this fact.

MMC is an alkylating agent that inhibits cellular division by inhibiting DNA and cellular RNA. It prevents the proliferation of underlying fibro-vascular tissue due to its anti proliferative effect on fibroblasts[20,21], induction of fibroblasts apoptosis[21] and the regulation of intracellular protein expression on mRNA levels[22]. MMC is also known for its potent antiangiogenic properties and that explains the suppression of new blood vessel formation at the wound site by MMC.[17]

Nevertheless, the effect of MMC on the conjunctival inflammation is not well known. Chang et al[12], proved that subconjunctival injection of MMC inhibits fibrovascular activity, reducing pterygium vascularization as well. Seet and associates[16] found that MMC reduces collagen deposition and suppress inflammation in glaucoma filtration surgery. Jain and associates[23] noticed that MMC decreases conjunctival inflammation in eyes with allergic conjunctivitis, but no previous works have studied the effect of MMC on pterygium regarding conjunctival inflammation after surgery.

This study presents, for the first time, the positive effect of MMC on the clinical conjunctival inflammation after CLAU surgery by comparing the inflammatory events between MMC + and MMC- groups. The eyes receiving intraoperative MMC seems to have lower number of conjunctival inflammatory events after surgery. Our results showed a lower incidence of inflammatory events at 1 month and 6 months after surgery in MMC+ (28.5% and 0% respectively) that those reported by Solomon et al[15] (31.5%) 3 month after extensive pterygium surgery using amniotic membrane transplantation without MMC, and those by

Keirkhah[3] and associates (40.7 %) for conjunctival inflammation using amniotic membrane transplantation with MMC application at 1 month after surgery. This study shows the incidence of inflammatory events is lower not just 1 month after the procedure but also after 6 months at MMC+. Although it is not the primary aim of our study, results confirm a statistical significant higher incidence of recurrences in MMC-. In our opinion, a higher incidence of inflammatory events may be related to a higher rate of recurrences. It seems that CLAU with MMC could be the better combination not only to reduce recurrences[8] but also inflammatory events.

Parallel to lower incidence of postoperative conjunctival inflammation, there was lower incidence of pyogenic granuloma after MMC+ compared with MMC- (2.9% vs 5.9%, respectively) 1 month after the surgery, although the difference was not significant, probably due to the few incidence disclosed. This lesion, which is inflammatory, is caused by exuberant healing with fibrovascular proliferation[24] and it is plausible to think that MMC could minimize the process. New studies with a large sample size could be necessaries to corroborate this hypothesis.

Despite of the important role of MMC in the treatment of the ocular diseases such as pterygium[11], penfingoid[26] and glaucoma[27] there are some possible complications, related with MMC effect on corneal epithelial healing[28]and stem cell toxicity. Persistent epithelial and endothelial defects and ischemic scleral necrosis have been reported after topical use.[29] To minimize MMC side effects and complications associated it is very important that the drug should be used in optimal concentration and exposure, and patients be carefully selected[11], excluding those with ulcer predisposition, wound healing such as Sjögren syndrome, severe keratoconjunctivitis, pre-existing corneal endothelial problems or herpes keratitis.

Future studies with larger sample sizes are required to analyze if MMC also reduces incidence of uncommon inflammatory events and to evaluate the role of other factors such as age, gender, pterygium morphologic features and different surgical techniques in the incidence of such postoperative inflammation.

In conclusion, eyes receiving intraoperative MMC after CLAU seems to present less hyperemia and graft contraction after surgery than those that did not receive MMC as an adjuvant factor. Intraoperative MMC could be also associated with a lower recurrence rates.

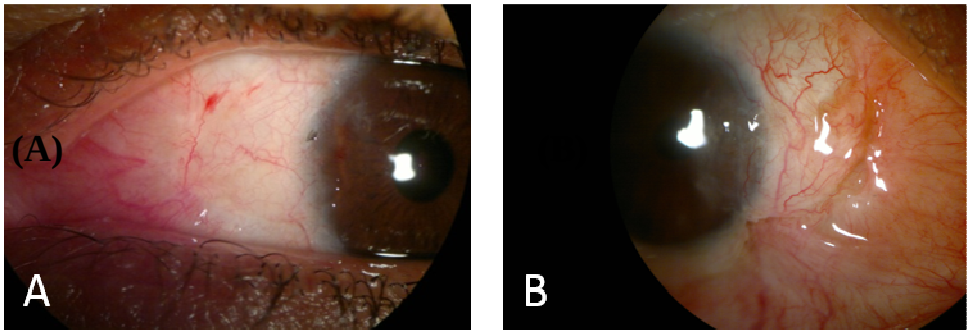

Figure 1. Absence (A) /Presence (B) of graft contraction 1 month after surgery. Note in image B the clear visualization of the graft limits and graft dehiscence with underlying bare sclera.

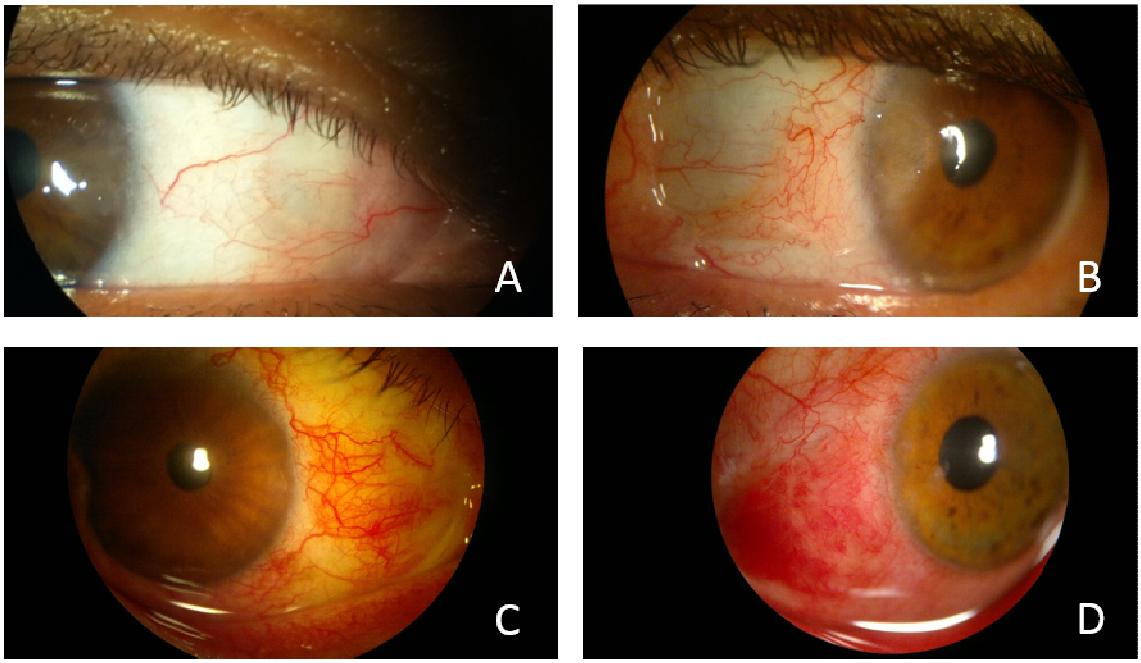

Figure 2. Postoperative conjunctival hyperemia. According to the degree of blood vessels injection, inflammation was graded as 0 (no hyperemia; A), grade 1 (mild B), grade 2 (moderate C) and grade 3 (severe D), in this case with presence of diffuse hemorrhage.

Preoperative Clinical Findings	MMC + Group		MMC - Group		p-value
	No.	%	No.	%	
Type of pterygium					0.480*
Primary	32	91.4	30	88.2	
Recurrent	3	8.6	4	11.8	
Pterygium morphology					0.560*
atrophic	6	17.1	9	26.5	
intermediate	19	54.3	18	52.9	
fleshy	10	28.6	7	20.6	
Race					0.135*
Caucasian	13	37.1	13	38.2	
Hispanic	16	45.7	20	58.8	
African	6	17.1	1	2.9	
Lesion area (mean ± SD) in mm^2	5.4 ± 3.9		5.8 ± 3.6		0.679**

Table 1 Preoperative Clinical Findings. ***** Fisher exact test. **Student t test. SD: standard deviation

Postoperative Clinical Findings	MMC + Group		MMC - Group		p-value
	No.	%	No.	%	
Presence of at least one complication	10	28.5	24	70.5	0.001*
Conjunctival hyperemia	9	25.7	22	64,7	0.001**
Graft contraction	1	2.9	20	47.1	0.001**
Conjunctival hemorrhages	4	2.9	7	26.5	0.230**
Conjunctival granuloma	1	2.9	2	5.9	0.614**

Table 2 Postoperative complications after LCAU with and without intraoperative MMC application. * Chi2 test. **Fisher exact test.

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