Effects of mitomycin C and cyclosporine A on experimentally induced glaucoma filtration surgery in rabbit model

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SUMMARY

The present study aims at investigating the effect of topical administration of mitomycin C (MMC) used solely and combined with cyclosporine A (MMC+CsA) on inhibition of the granulation tissue proliferation in experimentally induced glaucoma filtration surgery (GFS) in rabbits. A total of 18 New Zealand breed rabbits, control, MMC, and MMC+CsA groups of 6 subjects in each were used in the study. Clinical signs and complications in GFS region were evaluated using ophthalmoscopy, Schirmer tear test and tonometry 7 times at predetermined intervals between days 0-35. The right eyes of all subjects euthanized at the end of the study were harvested and histopathologically evaluated. According to clinical findings, GFS remained patent in all group subjects during the study except two subjects in the control group. Histopathological examination revealed marked cellular activities around GFS of the control group subjects as compared to the MMC and MMC+CsA groups. In this respect, no difference exists between two experimental groups. It has been observed that the intraocular pressure (IOP) value significantly decreased ($P<0.005$) in all groups on the day 5, which continued until the day 25 in control and MMC groups, and until the day 20 in the MMC+CsA group. The change in tear production rate showed to be more stable in the MMC+CsA than in the control and MMC groups. MMC and MMC+CsA are determined to have inhibited successfully granulation tissue proliferation around GFS and no marked difference was found between them as compared to the control group. It is suggested that in order to observe difference between these two groups, a more prolonged study is required.

Keywords: rabbit, glaucoma filtration surgery, mitomycin C, cyclosporine A

RÉSUMÉ

Effets de la mitomycine C et de la cyclosporine A sur le glaucome expérimentalement induit chez le lapin

Cette étude vise à étudier l'effet de l'administration topique de mitomycine C (MMC) seule ou associée à la cyclosporine A (MMC + CsA) sur l'inhibition de la prolifération des tissus de granulation sur glaucome de filtration chirurgicalement induit (GFS) chez le lapin. Un total de 18 lapins de la race Nouvelle-Zélande a été réparti en 3 groupes de six : témoins, traités MMC et traités MMC + CsA. Les signes cliniques et complications ont été évalués en utilisant l'ophtalmoscopie, le test de Schirmer et des mesures tonométriques à 7 intervalles prédéterminés entre 0-35 jours. Les animaux ont été euthanasiés en fin d'étude. L'œil droit de chaque animal a été récolté et évalué en histopathologie. Selon les résultats cliniques, le GFS est resté constant dans tous les animaux au cours de l'étude, à l'exception de deux animaux du groupe témoin. L'examen histopathologique n'a pas révélé de différenceentre témoin et traités par MMC ou MMC + CsA. La pression intraoculaire (PIO) a diminué significativement ($P <0,005$) dans tous les groupes le jour 5, puis suivi des évolutions variables dans les groupes témoins, MMC, et MMC + CsA. La production de larmes s'est montrée plus stables dans le groupe MMC + CsA que dans les groupes contrôle et MMC. Ces résultats suggèrent que des études sur une durée plus longue sont nécessaires pour analyser les différences entre groupes MMC et MMC + CsA.

Mots-clés : ophtalmologie, lapin, glaucome, mitomycine C, cyclosporine A

Introduction

Glaucoma is a common and slowly progressing eye disorder characterized by high IOP. It appears in the form of optic neuropathy which leads to the death of retinal ganglion cells and optic nerve axons [15, 22, 24]. Glaucoma requires immediate intervention due to the lack of treatment to eliminate neural cell damage and prevent the loss of vision [1, 22]. The main objective of the current glaucoma therapies is to protect the visual field by preventing damage to the optic nerve and retinal ganglion cells via lowering IOP [27]. The treatment of glaucoma includes medical treatment and non-invasive (laser) and invasive (penetrating) surgeries [22, 34, 41]. Medical treatment is proposed at the beginning as well as in mild cases [24, 34]. The laser, a non-invasive surgical intervention in glaucoma treatment, is considered an option between medical and invasive surgical treatments [22]. Invasive surgical methods become unavoidable in conditions in which medical and non-invasive surgical procedures fail to control high IOP [23, 35]. Invasive surgical technique is an attempt to create an alternative pathway for aqueous humor drainage blocked due to impaired trabecular meshwork [22]. Nowadays, most of the experimental and clinical researches on glaucoma are concentrated on two main techniques, such as anterior cameral shunt [5, 25] and glaucoma filtration surgery (GFS, trabeculectomy) [8, 32] to the long-term or permanent control of IOP [4, 14, 15, 20, 22, 23, 29, 30, 33, 34, 35, 42]. GFS used in the present study is based on a principle to establish an alternative drainage pathway (tunnel, fistula) from the anterior camera to the subconjunctival space to

facilitate aqueous humor drainage [33, 35, 38, 43]. The drainage of aqueous humor from the anterior camera to the subconjunctival space established with GFS can be clinically understood with the formation of a filtration bleb (bubble) under conjunctiva at the surgery site [5, 8, 25, 32, 36]. It has been reported that GFS may postoperatively be occluded by fibroblast proliferation and subconjunctival fibrosis (scar) played a leading role by episclera [7, 18, 23, 31, 33, 35]. Maintaining GFS function depends very much on the inhibition of intraluminal scar tissue development [35]. It has been suggested that this inhibition process can be managed by antiinflammatory (steroids) and antimetabolite (antiproliferative) agents with antifibroblastic properties. Well-known antimetabolites are 5-FU and MMC [14, 23, 29, 35] but due to some difficulties, especially in application frequency of 5-FU, MMC is nowadays more frequently used [26]. Antiproliferative agents, despite their critical importance in increasing the success rate of GFS, may induce significant ocular complications because of improper dose, time and frequency of use [14, 17, 35]. The success of GFS can be extended gradually by increasing the dose of an antiproliferative agent; however, such an application may induce drug-related toxicity which is claimed to be reduced with the simultaneous use of agents with different mechanisms of actions [23].

Cyclosporine-A (CsA) is commonly used in the treatment of many inflammatory eye diseases as an immunomodulatory and anti-inflammatory agent [2, 30]. Unlike other anti-inflammatory agents including corticosteroids, topical CsA improves ocular surface symptoms [9] with minimal adverse effects [28, 39, 40]. The antimetabolite properties of this agent in GFS have been investigated in some prospective, randomized clinical trials [9, 39, 40]. Fakhraie et al [9] in their study in glaucomatous patients who underwent GFS and received intraoperative MMC or 5-FU, reported that topical CsA had no effect on postoperative bleb function and IOP. A prospective, randomized clinical trial on antimetabolite properties of CsA in GFS found positive response and suggested more studies to substantiate its adjuvant role in GFS [39, 40]. Considering its broad therapeutic properties, including immunomodulatory, anti-inflammatory, immunosupressive and antimetabolite activities, and the low incidence of its adverse effects on the eye we hypothesized that topical CsA can potentiate antiproliferative effect of MMC when applied postoperatively as an adjuvant treatment. As a result, repeated postoperative usage of MMC might be prevented [14, 17, 23, 35] and hence drug-related toxicity reduced [23, 28].

The objective of this study was to assess whether topical CsA administration can improve postoperative antiproliferative action and late complications such as bleb leakage, infection, hypotonia and endophthalmitis of MMC following GFC using clinical and histopathological evaluation.

Materials and methods

The material of the study consisted of 18 adult female New Zealand breed rabbits weighting between 2-3 kg. The study received formal approval from Animal Experiments and Ethics Board of Firat University *(Protocol Number: 2011/09-119)*. Rabbits were fed with commercial feed, had free access to tap water, and were housed in an air-conditioned room (temperature: 23°C, humidity: 60%) with a 12-hour light–dark cycle.

Prior to the study, all subjects underwent thorough clinical, ophthalmoscopic and slit-lamp biomicroscopic examinations and their baseline IOP values and basal tear quantities Schirmer tear test (STT) were recorded. Following anesthesia of the subjects with i.m. 15 mg/kg of xylazine hydrochloride and 50 mg/kg of ketamine hydrochloride the right eyes of the subjects were prepared for aseptic surgery. The eyeball was fixed to dorsal and ventral eyelids with two sutures (5-0 silk, Dogsan, Turkey). A limbus base conjunctival flap of approximately 4x4 mm in size was performed between lateral and dorsal rectus muscles and the sclera was exposed. At this stage, the subjects were randomly divided into control, MMC and MMC + CsA groups of 6 animals in each. In the control group, the subjects received no application; however, in MMC and MMC+CsA groups the exposed sclera was treated first with MMC (0.4 mg/ml Mitomycin-C, Kyowa, Japan) impregnated nitrocellulose filter paper (nitrocellulose/filter paper sandwich, Invitrogen, USA) of approximately 4x4 mm in size for 5 minutes (Figure 1) and then the region was thoroughly washed with formal saline.

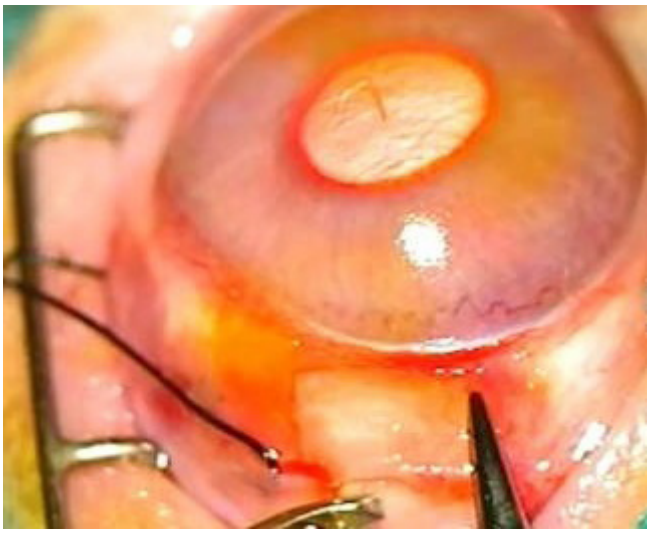


Figure 1: MMC impregnated nitrocellulose filter paper is placed in GFS region and covered with the conjunctival flap.

In all groups, a GFS tunnel was created with a 22 gauge sterile cannula about 1 mm behind the limbus from the sclera to the anterior chamber angle (Figure 2). The entrance of the tunnel was slightly widened (Figure 3) in order to readily localize the tunnel site at necropsy and facilitate dissection for histopathological examination. The conjunctival flap was fixed in place with separate horizontal U stitches as watertight manner using 8-0 absorbable synthetic suture material

(Surgicryl, SMI, Belgium) (Figure 4). Postoperatively the right eyes of control and MMC group subjects received 1 drop (0.1 ml) formal saline while MMC+CsA group subjects were instilled 1 drop (0.1 ml) 2% CsA containing solution (Restasis, Abdi Ibrahim), twice a day for a month. Additionally, all subjects were administered 1 drop topical antibiotic (Lomefloxacin, Okacin, Novartis) twice a day daily for a week.

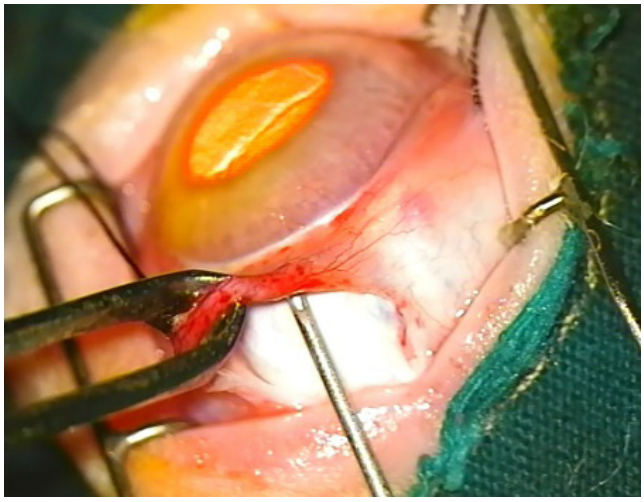


Figure 2: Creation of GFS tunnel toward the anterior cameral angle. CsA group, 3rd subject.

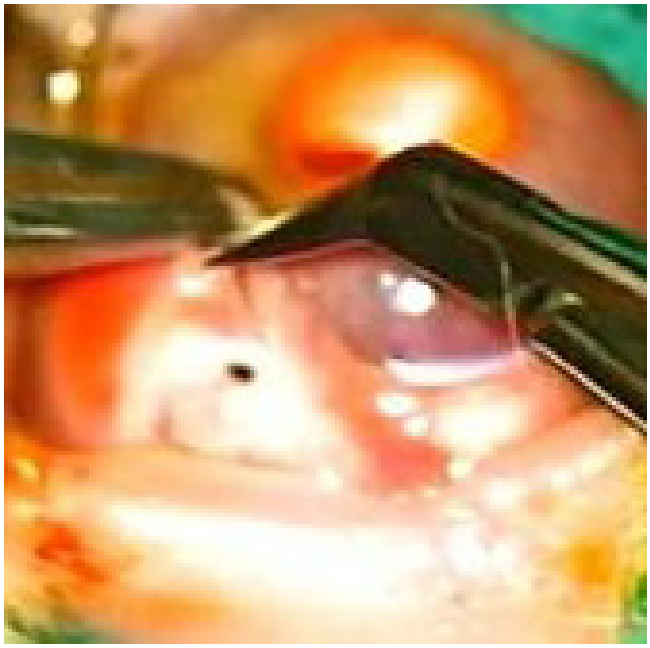


Figure 3: Last appearance of the tunnel. MMC group, 2nd subject.

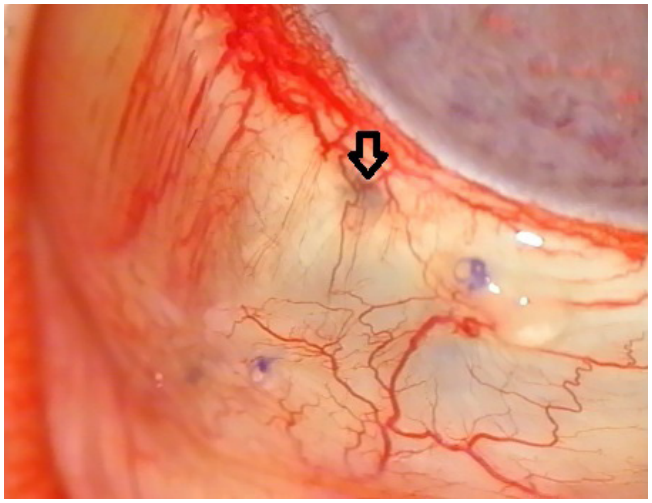


Figure 4: Operation microscopic view of GFS tunnel (arrow) in control group subject on day 5. Sutures (a) are visible. Bleb is diffuse, vascularized and shallow.

The study continued for 35 days, all ophthalmic examinations and measurements were carried out on days 0 (baseline), 5, 10, 15, 20, 25 and 35. All procedures were performed between p.m. 1^{00}-5^{00} to remove the effect of circadian rhythms on ocular parameters such as tear production and IOP. At these times, existence and properties of GFS tunnel and bleb (Table I), wound dehiscence, aqueous humor leakage, conjunctival hyperemia, subconjunctival bleeding, cornea edema and pupillar light reflex were evaluated. Conjunctival and corneal impairments were also assessed by using fluorescein dye under a slit-lamp biomicroscope with cobalt blue filter (XL-1, Shin-Nippon, Japan). Tear production rate was measured with STT (Tear Flo, Rose Stone Enterprises, India), IOP value with Tonopen Vet (Reichert, U.S.A.) and the data were recorded. At the end of the study all subjects were euthanized with intracardiac lethal dose of 10% thiopental sodium (Sodium pentothal, Abbott) and their right eyes were harvested. These samples were processed pursuing routine histological procedures, stained with Hematoxylin-Eosin and Masson's Trichrome (Biostain, U.K.) and examined under the light microscope (Nikon Ni-E, Japan). In all groups, the goblet cell density was evaluated in five microscope fields randomly distributed. The cell density was rated as follows: between 1-3 cells was estimated as low (+), between 3-6 cells as medium (+ +), 6 and above as high (+ + +). Cellularity (lymphocytes and eosinophils) was graded as + (>5 cells), ++ (<5-10> cells) and +++ (<10 cells). Collagen density and edema, found in less than 20% of the microscope field was rated as +, in 20 to 50% as ++ and in 50% and above as +++ .

Data were recorded as mean and standard deviation. Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA) version. The differences within a group and between the groups were determined with Post hoc test and those between which groups and which measurement times (days 0, 5, 10, 15, 20, 25 and 35) using Tukey and Duncan tests. Histopathological data were evaluated with a

one-way ANOVA. It was set to $P<0.005$ and $P<0.05$ at 95% confidence interval as statistically significant.

Results

During the study, a mild conjunctivitis was determined in 2 subjects in the control group and 1 subject in the MMC group on the day 15 and in 3 subjects in the MMC group on the day 20. No local or systemic infection, bleb rupture, humor aqueous leakage, corneal lesions, blindness and bleeding were encountered in any cases.

In the first examination after the surgery, GFS tunnel hole was evident in all control and MMC+CsA and in 5 MMC group subjects (Figure 4), and at the end of study it was seen in 3, 4, and 5 subjects in the control, MMC and MMC + CsA groups, respectively (Table I). Bleb was apparent in all groups throughout the study except 1 subject in control on day 35. Partial vascularized and vascularized type blebs (Figure 4) were common in control while vascularized (Figure 5) and partially vascularized types (Figure 6, 7) in MMC and MMC+CsA groups. In all three groups, broad (diffuse) type blebs (Figures 4, 7) were more common than localized (Figure 5) and shallow types (Figures 4, 7) more than bulged ones (Figure 5, Table 1).

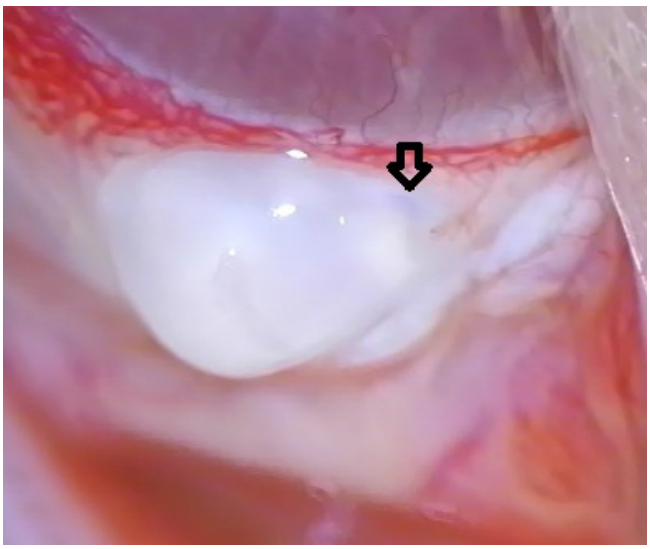


Figure 5: Operation microscopic view of a subject in MMC+CsA group on day 20. GFS tunnel (arrow) is hardly visible; bleb is localized (arrowhead), bulged and avascularized.

Group	MT	SN	TV	Existence and properties of blebs							
					Exist						
				Non-exist	Avascular	Partially vascularized	Vascularized	Broad	Localized	Shallow	Bulged
Control	5	6	6	-	-	1	5	4	2	4	2
	10	6	6	-	-	1	5	4	2	4	2
	15	6	3	-	-	2	4	4	2	5	1
	20	6	4	-	-	2	4	4	2	5	1
	25	5	3	-	1	3	1	4	1	5	-
	35	5	3	1	1	3	-	4	1	4	1
MMC	5	6	5	-	2	2	2	3	3	3	3
	10	5	5	-	1	2	2	3	2	3	2
	15	5	4	-	2	2	1	3	2	3	2
	20	5	3	-	1	4	-	3	2	3	2
	25	5	3	-	1	4	-	3	2	4	1
	35	4	4	-	1	3	-	3	1	3	1
MMC+CsA	5	6	6	-	2	4	-	4	2	4	2
	10	6	6	-	2	4	-	4	2	4	2
	15	6	6	-	2	3	1	4	2	4	2
	20	5	3	-	2	3	-	5	-	4	1
	25	5	4	-	4	1	-	5	-	4	1
	35	5	5	-	4	1	-	5	-	4	1

*Blebs were evaluated with direct and ophthalmoscopic examinations as well as utilizing biomicroscopic photographs and graded according to the following criteria: Vascularization (avascular: no vascularization on and around the bleb; partially vascularized: marked vascularization just around the bleb; vascularized: marked vascularization on and around the bleb), Diffusiveness (localized: bleb exceeded little the tunnel; broad: the bleb well-spread around the tunnel) and Deepness (shallow: tunnel is clearly visible; bulged: the tunnel is hardly visible or invisible)

Table I: Evaluation of GFS tunnel visibility (TV) and bleb clinical characteristics in terms of group, measurement times (MT) and the number of subjects (SN).

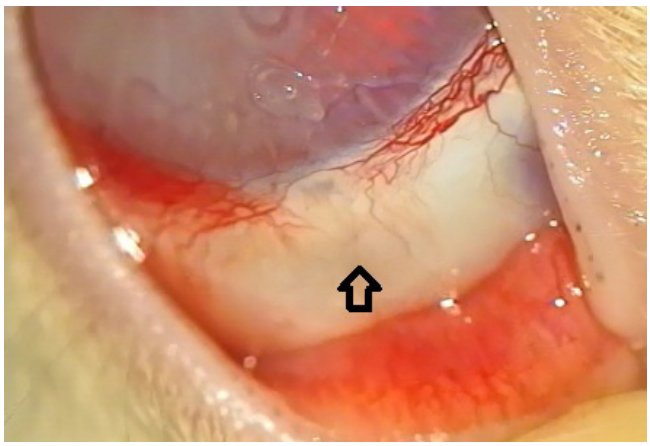


Figure 6: Operation microscopic view of a subject in MMC group on day 35. GFS tunnel (arrow) is marked; bleb is broad, bulged, and partially vascularized.

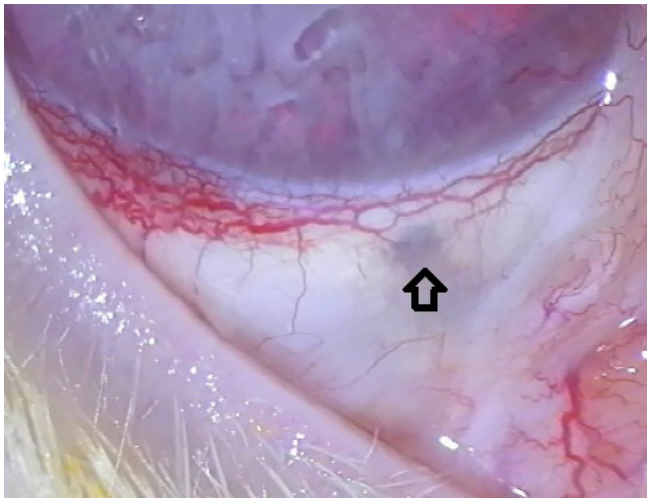


Figure 7: Operation microscopic view of a subject in MMC group on day 25. GFS tunnel (arrow) is apparent; bleb is diffuse, shallow, and partially vascularized.

There was a significant (P<0.005) difference in IOP data when each group data were compared within itself considering measurement time variable (Table 2). This difference was between days 0-5, 0-10, 0-15, 0-20 and 0-25 in the control group; between days 0-5, 0-15, 0-20 and 0-25 in MMC group and between days 0-5, 0-10, 0-15, 0-20 and 5-35 in MMC+CsA group. When evaluating minute tear production data in terms of measurement times, a significant difference was found in all groups. Pair comparison revealed that this difference was between days 0-25, 5-15, 5-20 (P<0.05), 5-25, 10-15, 10-20, 10-25 (P<0.005) and 25-35 (P<0.05) in control group and the days 0-5, 5-20, 5-25 (P<0.05) in MMC group and the days (P<0.05) 0-25 in MMC+CsA group (Table II).

Histopathological evaluation of GFS tunnel field determined that cellularity, collagen density and edema were more marked in the control group than MMC and MMC + CsA groups. In terms of these data no significant difference was observed between MMC and MMC + CsA groups (P>0.05). Additionally, an increase in hyper cellular fibrosis and goblet cell density as well as a mild inflammatory response were detected in the subconjunctival areas of control subjects (Table III, Figure 8-9).

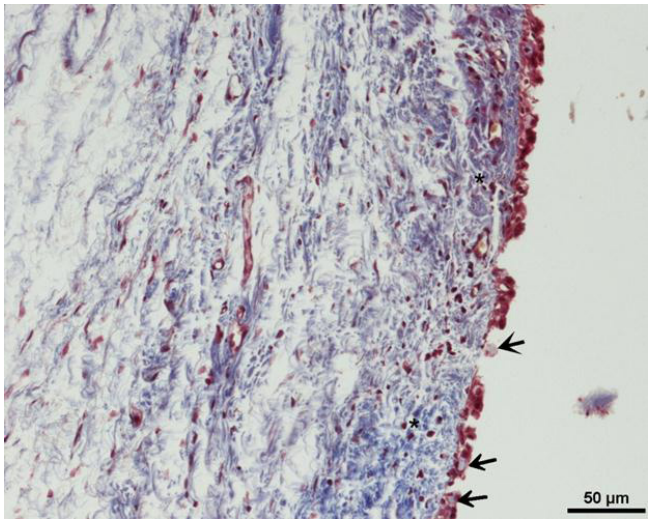


Figure 8: Dense collagenous fibrotic tissue growth (*), more goblet cells (arrow) and marked edema in subconjunctival area. Control group, Masson Trichrome.

Variables	Groups	Measurement Times							*P*
		0	5	10	15	20	25	35	
IOP *(mmHg)*	**Control**	15.13±2.14^{a}	9.54±1.74^{b}	9.85±1.68^{b}	10.71±1.71^{b}	10.50±1.16^{b}	10.65±2.21^{b}	12.85±1.99^{ab}	<0.005
	MMC	12.08±1.66^{a}	8.41±1.47^{b}	8.90±1.85^{ab}	8.40±1.15^{b}	8.85±2.00^{b}	9.60±0.80^{b}	10.57±1.40^{ab}	<0.005
	MMC+CsA	13.38±2.02^{a}	7.33±1.69^{b}	8.71±2.15^{bc}	8.67±2.07^{bc}	8.75±2.29^{bc}	10.25±2.42^{abc}	11.55±1.6^{ac}	<0.005
STT *(mm/dk)*	**Control**	4.50±2.00^{ac}	3.83±0.93^{c}	3.33±1.21^{c}	4.00±1.26^{ab}	4.33±0.82^{ab}	5.20±1.30^{b}	4.40±1.52^{ac}	<0.005
	MMC	4.22±1.11^{a}	4.08±1.02^{b}	4.20±1.30^{ab}	4.60±1.52^{ab}	5.60±1.34^{a}	5.60±0.89^{a}	3.75±0.50^{ab}	<0.05
	MMC+CsA	4.28±0.92^{a}	5.00±1.67^{ab}	5.67±1.37^{ab}	4.17±1.17^{ab}	5.40±1.14^{ab}	6.20±1.30^{b}	4.60±0.55^{ab}	<0.05

*The difference between groups in the same row with different letters are statistically significant (P < 0.05)

Table II: Statistical evaluation of mean data of IOP and tear production quantities in three groups in terms of measurement time factor.

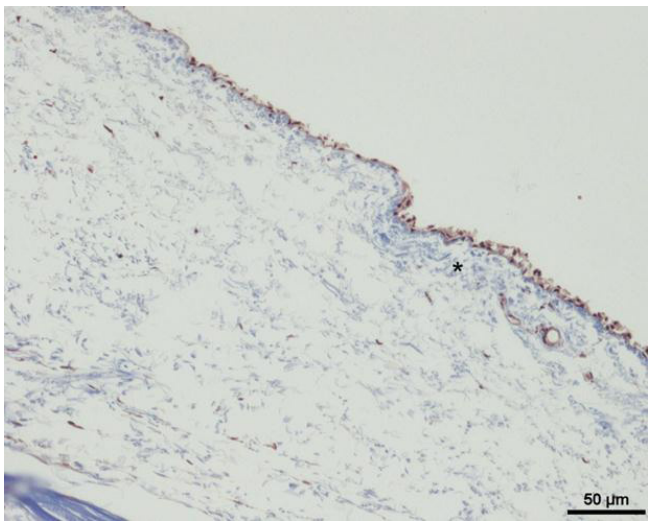


Figure 9: Mild collogenous fibrotic tissue density (*) and edema in subconjuctival area. MMC+CsA group, Masson Trichrome.

Discussion

In the first examination after the operation, GFS tunnel was visible in all subjects except one in MMC group. At the end of the study it was seen that occlusion process had started in the tunnel lumens of just two control group subjects. The rapid development of scar tissue appears to be effective on this process [7, 18, 23, 31, 33, 35]. Histological examination showed a dense cellularity and collagen deposition in GFS fields and hypercellular fibrosis tissue growth and goblet cell density in subconjunctival zones in control group compared to MMC and MMC + CsA groups (Table III). In this respect, no significant change was exist in last two groups. It is reported that CsA promotes goblet cell proliferation [21], whereas MMC reduces cellularity and collagen accumulation [20, 23]. The findings of the present study support the opinion [20, 23] concerning the effects of MMC, contrary to that related to CsA action [21]. That action of CsA may be suppressed by antiproliferative effect of MMC [16] in the group where MMC was used in combination with CsA.

When the vascularization characteristic of bleb throughout the study was evaluated in total with regard to group variability, it was observed that partially or vascularized types were more common in the control group contrary to avascularized and partially vascularized types in the MMC and MMC + CsA groups. It is thought that the suppression of cellular proliferation via antimetabolite property of MMC [3, 19] and immunosuppressive properties of MMC [3] and CsA [37] may prevent the bleb to undergo new vascularization process in two experimental groups unlike in the control one.

GFS is characterized by formation of subconjunctival bleb [6, 36] which is understood by subconjunctival liquid accumulation of diffuse, localized, shallow or bulged in patterns in the area where the fistula is formed [6, 27]. In all three groups, diffuse type blebs were more common than localized and of the shallow type than of the bulged one. In clinical glaucomatous cases, however, the bulged type is more commonly defined [5, 6]. In such cases, a significant portion of aqueous humor drains out from GFS tunnel to subconjunctival space due to impaired trabecular meshwork drainage [10]. Here GFS was carried out on healthy subjects with intact drain system. Thus, most of aqueous humor preferentially drains out via unimpaired trabecular meshwork. The portion that drained via scleral tunnel to subconjunctival space appeared not high enough to cause bulged type blebs.

Occlusion of GFS tunnel with fibroblast proliferation or scleral fibrosis leads to the failure of the surgery [7, 18, 23, 31, 33, 35, 36]. Many agents with antiproliferative or antiinflammatory features are used alone or in combination to prevent this development leading to occlusion of the GFS tunnel [3, 8, 9, 25, 32]. In this study, the growth-inhibiting effects of CsA and MMC on scar tissue were studied with successful results in the MMC and MMC+CsA groups as compared to the control, however no significant difference between them when clinical and histopathological findings and data of IOP as well as tear production were evaluated.

Ultimate care is paid to minimize tissue damage and bleeding during GFS to reduce the rate of fibroblastic formation [13, 23, 43]. Postoperative use of anti-inflammatory drugs also contributes positively to this process. However, these measures fail to prevent fibroblastic formation in long-term [23]. This is also true for GFS supported with 5-FU [38] and MMC [11]. The current study used CsA with the purpose to potentiate the antiproliferative effect of the MMC in long-term benefiting from its anti-inflammatory, immunomodulatory, immunosuppressive and antiproliferative effects [4, 37, 39, 40]. In groups where MMC were used alone or in combination with CsA, histopathological examination determined that GFS tunnel

Parameters	Groups		
	Control	MMC	MMC+CsA
Cellularities	2.71±0.18[a]	1.42±0.20[b]	1.28±0.18[b]
Collagen and edema	2.85±0.14[a]	1.28±0.18[b]	1.14±0.14[b]
Goblet cells	2.57±0.20[a]	1.14±0.14[b]	1.00±0.00[b]

*The difference between groups in the same row with different letters is statistically significant ($P < 0.05$).

Table III:. Statistical evaluation of histopathologic parameters in GFS field in terms of group factor.

hole maintained patency along the study with no fibroblastic activity around the tunnel. However, from the present findings, it is difficult to judge whether or not CsA has any fibroblastic activity [39, 40] and it is suggested that a long-term study [23] with an additional use of a group including only CSA is required for further clarification.

Suppression of fibroblastic activity after GFS is a prerequisite in ensuring a lifelong survival of the bleb and permanent control of IOP [23]. Although the use of antiproliferative agents is critical for increasing the success rate of GFS, inappropriate dose, time and frequency of use can lead to significant ocular complications [14, 17, 23]. The effect of antiproliferative agents on bleb can be extended by increasing incrementally their doses, but this practice may lead to an increase in drug-related toxicity in the eye [22]. Drug-related toxicity can be reduced by the combined use of agents having different mechanism of actions [8, 9, 23, 25, 32]. To date, 5-FU and MMC are commonly used antiproliferative agents [20, 23] in GFS and in the present study MMC was tried. Because of the specified risk of repeated doses, it is suggested [16] that MMC should be combined with alternative agents to prolong the survival rate of GFS. The investigation of novel agents with antiproliferative action is continues [5, 8, 9, 25, 32, 39, 40]. The present study utilized CsA in an assumption to extend antifibrotic action of MMC [9, 39, 40] and to reduce its side effects resulting from repeated postoperative applications [14, 22, 23, 42]. The current IOP data are coherent with those of the study of Buttler et al [5] showing that CsA may not have antifibrotic effect. At the opposite, histopathological findings by Turacli et al [39, 40] suggest the presence of such an activity.

The functionality of GFS fistula in glaucomatous patients depends on the duration of inhibition of granulation tissue [7, 23]. In the present study, this inhibition process was maintained by MMC alone and by a MMC+CsA combination. According to clinical and histopathological results, the tunnel lumens belonging to the MMC and MMC+CsA groups maintained patency throughout the study in all subjects with no significant difference between them. A significant decrease detected in IOP measurements in all group subjects at the beginning of the study is consistent, but the rise of IOP to baseline level towards the end of the work conflicts with clinical and histopathological findings. This situation is thought to result from minor fluctuation in IOP owing to drainage of aqueous humor via the regular channels of trabecular angle no more needed for tunnel drainage because the subjects used in the study, unlike glaucomatous individuals [15, 22, 24], had functional trabecular angle. More stable fluctuation in the amount of tear production parameters of the MMC+CsA group can be explained with the modulating activity of CsA on tear production [2].

In conclusion, MMC and MMC+CsA successfully inhibited granulation tissue proliferation and thus prevent tunnel closure during the period in which the study was conducted with no important difference between both groups. It was suggested that in order to determine a marked difference between these two groups regarding the parameters, a more prolonged rabbit model would be consisted of and just CsA would be added, is required in the future.

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