

Effects of 5-Fluorouracil and Mitomycine-C on Glaucoma Filtration Surgery in Rabbits

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Abstract: The purpose of this study was to examine the effects of 5-fluorouracil (5-FU) and mytomycine-C (MMC) on the success rate of glaucoma filtration surgery (GFS). A superior fornix-based conjunctival flap was created on the right eyes of 12 rabbits divided randomly into three equal groups termed groups 1, 2 and 3. After a small piece of sponge soaked with balanced salt solution (BSS) in group 1 (control), 5-FU (50 mg/ml) in group 2 and MMC (0.02 mg/ml) in group 3 was kept on the filtration sites for 5 minutes, the filtration fistula (sclerectomy) was performed and the flap was replaced. Postoperatively, BSS, 5-FU and MMC with doses of 0.1 ml were injected 4 times with certain intervals into the inferior fornixes of the same groups. The presence of the corneal edema, neovascularization and defect, appearance of the filtering bleb, states of the aqueous humor, iris, lens and changes in intraocular pressure (IOP) were observed. In one case, severe conjunctival hyperemia associated with the corneal flush and hyphema was determined. A mild corneal edema in half the members of group 1, and all the members of groups 2 and 3, was found. The IOP, which reduced sharply in all cases immediately after the GFS, reached its preoperative level approximately in 14, 28 and 51 days in groups 1, 2 and 3, respectively. There was a significant difference ($P<0.001$) both within and between the groups regarding the survival rate of the filtering fistula. The duration of failure of the filtration bleb was also found to be short in group 1 when compared with group 2 and in group 2 when compared with group 3. In conclusion, the success rate of the GFS was determined to be significantly higher ($P<0.001$) in MMC than 5-FU and in 5-FU than in the control. Additionally, it was observed that both MMC and 5-FU produced no persistent adverse effects on the eye tissues.

Key Words: Antiproliferatives, Glaucoma Filtration Surgery, Rabbit.

Tavşanlarda Glakom Filtrasyon Operasyonu Üzerine 5-Fluorourasil ve Mitomycine-C'nin Etkileri

Özet: Bu çalışmada glakom filtrasyon cerrahisi'nin (GFC) başarısı üzerine 5-fluorourasil (5-FU) ve mytomycine-C'nin (MMC) etkilerinin incelenmesi amaçlandı. Grup 1, 2 ve 3 olarak adlandırılan üç eşit gruba rasgele ayrılan 12 tavşanın sağ gözlerinde dorsal fornix kaideli konjunktival flap oluşturuldu. Grup 1'de serum fizyolojik, grup 2'de 5-FU (50 mg/ml) ve grup 3'te ise MMC (0,02 mg/ml) ile ıslatılmış küçük bir sünger parçası filtrasyon bölgesi üzerine 5 dakikalık bir süreyle bekletildikten sonra filtrasyon fistülü (sklerektomi) gerçekleştirildi ve konjunktival flap yerine dikildi. Operasyondan sonra 0,1 ml dozlarında serum fizyolojik, 5-FU ve MMC aynı grupların inferior fornix'lerine belirli aralıklarla 4 kez uygulandılar. Kornea'da ödem, neovaskülarizasyon ve defektin varlığı, filtrasyon bleb'inin görünümü, humor aköz, iris, lens'in durumları ve göz içi basıncındaki değişiklikler incelendi. Bir olguda şiddetli konjunktival hiperemiyle birlikte korneal kızarıklık ve hyphema saptandı. Grup 1'deki olguların yarısında ve grup 2 ve 3'teki olguların tümünde hafif bir corneal ödem gözlemlendi. Operasyondan hemen sonra hızlı bir düşüş gösteren göz içi basıncı operasyon öncesi değerine, 1. 2. ve 3. grup olgularda sırasıyla yaklaşık olarak 14, 28 ve 51 günlerde ulaştı. Filtrasyon fistülünün yaşam süresi yönünden gerek grup içinde ve gruplar arasında belirgin bir fark ($P<0,001$) mevcuttu. Filtrasyon bleb'inin başarısızlıkla sonuçlanma süresi grup 2 ile karşılaştırıldığında grup 1'de ve grup 3 ile karşılaştırıldığında ise grup 2'de daha kısa olduğu gözlemlendi. Sonuç olarak GFC'nin başarı oranının, 5-FU'e göre MMC'de ve kontrol grubuna göre 5-FU'de belirgin bir şekilde daha yüksek ($P<0,001$) olduğu saptandı. Ayrıca MMC'nin ve 5-FU'in göz dokusu üzerine herhangi bir kalıcı yan etkisinin olmadığı gözlemlendi.

Anahtar Sözcükler: Antiproliferatifler, Glakom Filtrasyon Cerrahisi, Tavşan.

Introduction

Glaucoma is potentially a blinding disease. The primary goal of its therapy is to bring the increased intraocular pressure back to its normal level (1). For this

purpose, many medical and operative procedures are advocated (2, 3). However, surgical intervention usually warrants the long-term or permanent control of intraocular pressure (IOP) in most glaucomatous cases,

particularly with the small animals. Glaucoma filtering surgery (GFS) or trabeculectomy is a surgical technique designed to lower IOP by allowing the passage of aqueous humor from the anterior chamber to the subconjunctival space via a filtering fistula (sclerectomy) created in the sclera (4, 5). This fistula can be blocked by a rapidly progressing scar soon after the GFS unless required adjunct measures are taken into consideration (5, 6). Thus, inhibition of the scar formation is essential to enhance the chance of surgical success and to prolong the survival rate of the bleb (4). The agents, capable of delaying the scar formation, are claimed to increase the success rate of the GFS (4). For this purpose, many drugs including bleomycin (5), corticosteroids (7), 5-fluorouracil (8, 9), mitomycin-C (10, 11), taxol, etoposide (12), daunorubicin and anthracycline daunomycin (13) have been tried. Among them, 5-fluorouracil (5-FU), a pyrimidine analog, and mitomycin-C (MMC), an antibiotic with alkylating properties, are known as the drugs used commonly in both experimental and clinical studies (8, 9, 14). It has been noted that in case of wrong or aggressive use, these agents may cause a variety of complications, including the erosion and necrosis of the cornea, defect of the corneal epithelium, neovascularization of the cornea and iris, ulceration of the sclera and wound leakage of the conjunctiva (5, 13, 15). To lessen these complications, alternative doses, application routes and times related to these drugs have been researched. However, an ideal treatment protocol has not been found yet and the requirement for further studies has been emphasized (16). These drugs are administered subconjunctivally prior to and after the GFS and topically during the surgery. Even though these administration routes can be tried either singularly or together during a study, most workers appear to prefer the latter form (16, 17, 18). The purpose of this study was to examine the effects of 5-FU and MMC on the outcome of the GFS conducted on cases undergoing the same experimental design and treatment protocol. During this experiment, we tried to remain focused particularly on the influence of these drugs on the ocular tissues, IOP and bleb survival time.

Materials and Methods

The study was performed on twelve rabbits weighing between 1.4 and 2.4 kg. They were anesthetized with an i.m. injection of 10 mg/kg xylazine hydrochloride

(Rompun, Bayer) and 50 mg/kg ketamine hydrochloride (Ketanes, Alce). After the right eyes of these animals were prepared for aseptic surgery, a lid speculum was inserted and a superior fornix-based conjunctival flap was created at the 12 o'clock position (Figure 1). These animals were assigned randomly to 3 equal groups called groups 1 (control), 2 and 3. A cellulose sponge of about 5x5x5 mm in size, soaked with balanced salt solution (BSS) in group 1, 5-FU (50 mg/ml) (Roche) in group 2 and MMC (0.02 mg/ml) (Onko) in group 3, was kept between the conjunctival flap and sclera where the filtration fistula was conducted, for 5 minutes. The filtration sites treated with 5-FU and MMC were irrigated thoroughly with BSS and the filtration fistula (sclerectomy) was performed with a 1.5 mm biopsy punch (Figure 1). The conjunctival flap was secured in place so as to be watertight with simple interrupted sutures using 8-0 vicryl (Ethicon) (Figure 2). BSS, 5-FU



Figure 1. Showing an anterior fornix-based conjunctival flap and filtration fistula (arrow).



Figure 2. Conjunctival flap has been secured in place with simple interrupted sutures.

and MMC with doses of 0.1 ml were injected into the inferior fornix of the operated eyes on the postoperative days (PODs) 2, 5, 9 and 14 under anesthesia. Postoperatively, all cases received topically terramycin ophthalmic ointment (Pfizer) twice a day for a week. The presence of the corneal edema, flush, ulceration and defect, depth of the anterior chamber, state of the aqueous humor, appearance of the filtering blebs and presence of other unspecified lesions were investigated daily for the first two weeks and once a week afterwards. During this investigation, we benefitted from a pen torch, ophthalmoscope (SMIC, China), stereomicroscope (Nixon SMZ-T, Japan) and fluorescein test. IOP was measured with the schiottz tonometry (Riester, Germany) prior to the surgery, at PODs 2, 5, 9, 14 and then once a week up to the end of the study. To avoid bias, IOP was noted at each time as the mean value of three independent measurements taken at 5 minute intervals. Macroscopically, the bleb was evaluated as either functional (elevated and hyperemic) or non-functional (flat and pale). The drainage of the filtering fistula was considered to have failed when the bleb appeared flattened and IOP was just 2 mmHg below its value determined prior to the GFS. The follow-up period was around 10 weeks.

Both right and left globes of all cases, exsanguinated under deep pentobarbital anesthesia, were removed with the subtotal exenteration method and fixed in buffered formalin solution. Two samples, from the bleb sites (12 o'clock position) and from the area 180° from the bleb sites (6 o'clock position), were obtained from each eye. After these samples were processed using routine histologic procedures and stained with H-E, they were examined regarding the cellular infiltration of the conjunctiva and sclera, patent of the filtration fistula, lesion and thickness of the cornea and states of the iris and ciliary epithelium. The thicknesses of both right and left corneas were measured at 4 locations with equal intervals between the limbus and the center. The data obtained from the measurements of IOP and corneal thickness were analyzed with ANOVA. Results were considered to be significant at $P < 0.001$.

Results

During the follow-up period, problems like wound dehiscence, bleb rupture and adverse systemic reactions against the drugs were not determined. A mild corneal

edema (Figure 3), particularly adjacent to the filtration sites, was observed in half the cases in group 1 and all of the cases in groups 2 and 3. It was associated with severe conjunctival hyperemia, corneal flush near the limbus and hyphema in one of the cases in group 1 (Figure 4). The results of the fluorescein test were negative in all cases except one (Figure 5).

The IOP which reduced sharply in all groups after the GFS, persisted on average for 9 days in group 1, 21 days in group 2 and 42 days in group 3. Then it started to rise and reached its preoperative level approximately in 14, 28 and 51 days respectively in groups 1, 2 and 3. The lowest IOP measured here was 9.4 mmHg, which was determined in four rabbits, two from each of groups 2 and 3. It remained so up to POD 9 in both cases of group 2 and until POD 14 of one and POD 21 of another case of group 3.



Figure 3. In this photograph, corneal edema, especially adjacent to the operation site, is observed. The bleb elevated slightly over the sclera, is demarcated by a hyperemic area (arrow heads).

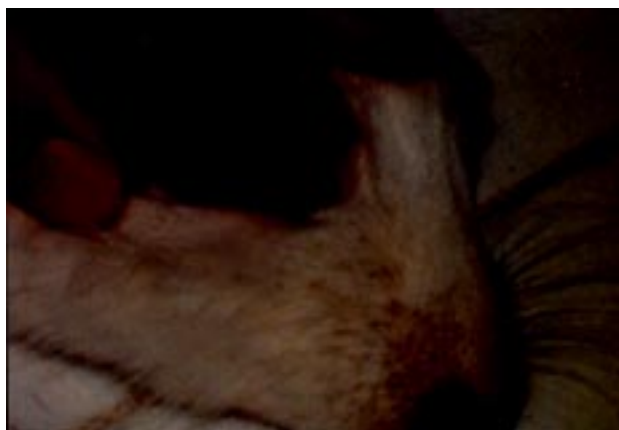


Figure 4. Conjunctival hyperemia and hyphema are seen.



Figure 5. The corneal surface is stained with fluorescein.

In a functional bleb, the conjunctiva overlaying the filtration fistula was distorted outwardly and the lumen of the fistula was hardly distinguished (Figures 3 and 6). In a non-functional bleb, the lumen of the fistula was clearly visible and the conjunctiva adhered tightly to the episclera. (Figure 7). The bleb survival rates of all groups are shown in the Table, which demonstrates that the duration of the bleb resulting in failure is significantly longer in group 3 than in group 2, which is longer than in group 1.



Figure 6. In this photograph, the bleb elevated over the sclera, appears hyperemic. The filtration fistula (arrow heads) is hardly visible due to the bleb formation.

Despite some exfoliation of the epithelial and endothelial layers of the cornea during the histologic processing, it appeared normal in most cases. A case in group 1 had an edematous cornea infiltrated by inflammatory cells and mostly empty blood vessels (Figure 8). In all groups, the lumens of the filtration

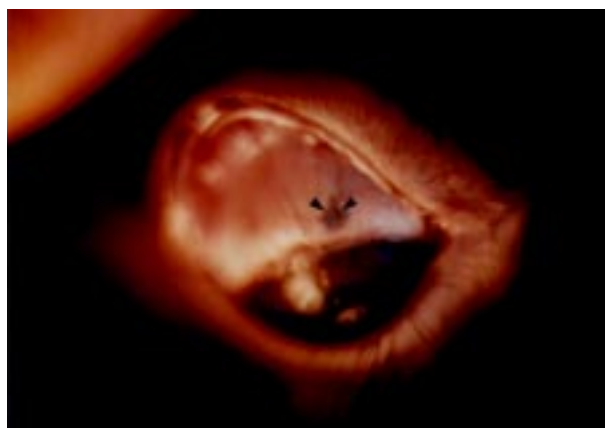


Figure 7. The bleb (arrow heads) became flattened and the conjunctival flap overlying the fistula merged fully with the sclera.

Table. Results of the bleb survival rate recorded in three groups during follow-up period.

Measurement days	Bleb survival rate (%)		
	Group 1 (Control)	Group 2 (5-FU)	Group 3 (MMC)
2	100	100	100
5	"	"	"
9	75	"	"
14	25	"	"
21	0	75	"
28	"	25	"
35	"	0	"
42	"	"	50
49	"	"	50
56	"	"	25
63	"	"	0

fistulas were sealed with a cicatrix tissue (Figure 9) that contained inflammatory cells in some cases (Figure 8). The inflammatory cells were also found in the iris and ciliary body of one case. When the data of the corneal thickness were evaluated, significant differences between the cases and locations were found (Figure 10). Mean corneal thickness was about 731 μm adjacent to the limbus and 438 μm at the center. The right corneas were determined to be slightly thicker than the left ones. In this regard, differences between groups were non-significant (Figure 10).

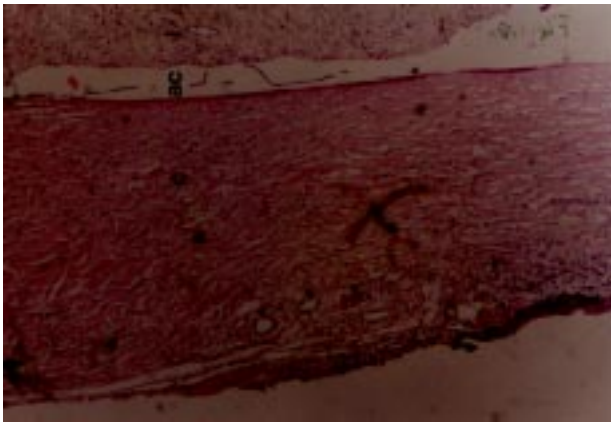


Figure 8. In this micrograph, anterior camera (ac) and iris (I) are visible. Epithelial and endothelial layers of the cornea (c) have exfoliated. Descemet's membrane appears intact. The cornea is edematous and contains inflammatory cells and mostly patent vessels. H-E, X20.

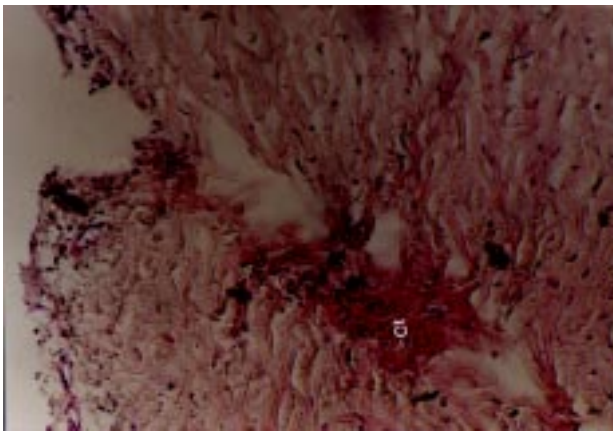


Figure 9. Longitudinally sectioned lumen of a filtration fistula is blocked partially with a cicatrix tissue (Ct). Some inflammatory cells are observed in and around this tissue (arrows). H-E, X20.

Discussion

A variety of antiproliferative agents have been tried to delay the early closure of the filtration fistula (5, 7, 9, 11, 12). They are administered subconjunctivally prior to or after the GFS, or topically to the filtration site during the surgery. However, there are some disputes on the benefits of these application routes. Some authors (16, 18, 19) conducted work to compare the outcomes of the subconjunctival and topical administrations or their combinations using MMC. They found that subconjunctival application prior to or after the GFS did not significantly prolong the survival rate of the filtration fistula as compared with the control. When the subconjunctival and topical intraoperative administrations were combined, a dramatic increase in the bleb survival rate was found to occur (7, 19). Despite the marked benefit of the combined treatment, the subconjunctival way is reported to have several limitations, i.e. discomfort to the patients, inconvenient for the physician, expose the eyes to septic complications, increase the rates of corneal epithelial defects and wound leakage (5, 14, 20). During the clinical examination of this study, edema in the cornea, particularly adjacent to the filtration sites was found. It was associated with severe conjunctival hyperemia and corneal flush in one of the cases in group 1. The observation of the edema in control group cases as well, rules out the possibility of the presence of any correlation between this and the adverse effects of the drugs. In this study, a problem like patient discomfort was not experienced, since the subconjunctival administration was carried out under anesthesia. This is believed to prevent the occurrence of any risk related to the drug application, as it allows them to be given the subconjunctiva comfortably.

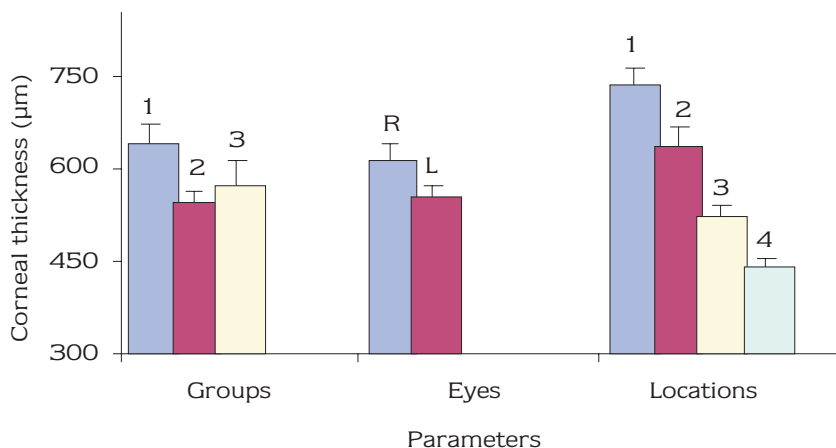


Figure 10. Regarding the corneal thickness, there is a significant difference between the 4 locations, but a non-significant difference between the right (R) and left (L) eyes and the three groups.

The studies on topical intraoperative MMC application, noted the bleb survival rate as 30 days in rabbits (16), 56 days in monkeys (19) and 21 days in dogs (21). These data show that various species respond differently to the same treatment protocol. This situation is thought to be due to differences in healing speeds between species. In the current study, the bleb survival rate in MMC treated cases is about 51 days, which is far greater than the value recorded by the previous study (16). This variation is believed to result from the treatment protocols of these two studies.

In this study, a sharp reduction in the IOP was detected in all groups soon after the GFS. The reduced IOP returned to its preoperative level in 14, 28 and 51 days respectively in groups 1, 2 and 3. It can be inferred from these parameters that the bleb survival rate of MMC is significantly higher than that of 5-FU, which is higher than that of the control group. This rate can be much higher in other species when the vigorous healing response of the rabbit wound is also taken into account (4, 22). There are some suggestions as to how MMC and 5-FU help to keep IOP low for a long time compared to the control (7, 11). First, they have antiproliferative effects on the ocular cells, which may delay the filtration fistula from being blocked by scarring (17). This is supported by the findings of the present study. Because, the blebs appear to survive longer in eyes treated with drugs compared to the control group. The second suggestion (4, 22) is that reduced IOP may persist due to the long-lasting toxic effects of these drugs on the cells of the ciliary process. The short episcleral application of MMC on the unoperated rabbit eyes was found to cause a decrease in the production of the humor aqueous (22). However, Jampel et al. (12) suppose that this reduction should be very low even if it is present. The absence of marked histological changes in the ciliary processes of the eyes treated with MMC and 5-FU in the present study supports the claims of the former authors (12). Additionally, the ocular signs seen in the eyes treated with them were transient, which indicates that MMC and 5-FU may not have serious toxic effects on the ocular tissue if they are used properly. Gressel et al. (23) in an experimental study on monkeys, reported that IOP declined in non-drug-treated control eyes after the GFS. An inhibition of the cellular secretion of the ciliary processes by subclinical postoperative inflammation is believed to cause this reduction. In this study,

postoperative inflammation was determined in some rabbit eyes, but no difference between the IOP values of the cases with and without ocular signs was found.

It has been reported that 5-FU enables the delaying of the conjunctival and scleral fibroblast outgrowths for one week more than the control. After the fibroblast outgrowths recovered, an abnormal growth rate and appearance were not observed (24). These findings are compatible with those of the current study, since no abnormal development in the scleratized sites during the histologic examination was determined. In contrast, it has been reported that the cell outgrowths in the tissues treated with MMC were significantly slower than those treated with distilled water in specimens taken 30 days after the surgery. Tissue culture experiments showed that the effects of 5-FU and MMC were largely correlated with their concentrations in, and exposure times to, the tissues (11). Some authors (25) found that the cellular proliferation of the rabbit ocular tissues stopped between 14 and 38 days depending on the types of, and their exposure times to, antiproliferatives. Khaw et al. (11) reported that 5-FU had short, but MMC had long-lasting effects on the ocular tissues. These findings conform with those of the current study, as the bleb survival rates of MMC and 5-FU were found to be respectively 28 and 51 days.

Regarding the data of the corneal thickness, there was a statistically significant difference between cases, but a non-significant difference between the groups. The cause of individual variations in the corneal thickness was reported to be due to factors like the age, sex and weight of the cases (26). The results of this study demonstrate that the cornea becomes thinner toward its center. Although the right and left corneas were found to differ in thickness, it was statistically non-significant. When the results of the control group were compared with those of the experimental groups, no difference was found between them. These findings also indicate that the drugs used here do not impose any marked side effect on the cornea.

The success rate of the GFS in MMC treated eyes was determined to be significantly higher than that for 5-FU. Therefore, the present authors agree with the suggestion of Khaw et al. (11) that 5-FU can be appropriate for cases requiring just temporary cessation of the healing processes of the ocular tissues while MMC can be appropriate for those which need either a temporary or

prolonged delay of the ocular healing processes. Additionally, these drugs appear to achieve these functions by producing no persistent adverse effects on the eye tissues. It was concluded that MMC and 5-FU could be considered as alternative drugs for the treatment of glaucoma in clinical cases.

References

1. Serle, J.B.: Pharmacological advances in the treatment of glaucoma. *Drugs Aging* 1994; 5, (3): 156-170.
2. Renwick, P.W.: Glaucoma. *Manual of small animal ophthalmology* (Eds. Jones SMP. and Crispin SM) BSAVA, Sussex. 193-211, 1993.
3. Akin, F. and Samsar, E.: Göz Hastalıkları, Tamer Matbaacılık, Ankara. 113-117, 1999.
4. Tilleul, P., Denis, P., Maignen, F., Elena, P.P., Nordmann, J.P., Leverage, R. and Rostene, W.: Effect of different formulation of mitoxantrone (solutions, nanospheres, liposomes) on glaucoma surgery in rabbits. *Ophthalmic Res.* 1997; 19, (4): 218-226.
5. Sakamoto, T., Oshima, Y., Sakamoto, M., Kawano, Y.I., Ishibashi, T., Inomata, H. and Ohnishi, Y.: Electroporation and bleomycine in glaucoma-filtering surgery. *Invest. Ophthalmol. Vis. Sci.* 1997, 38, (13): 2864-2868.
6. Doyle, J.W., Sherwood, M.B., Khaw, P.T., McGrory, S. and Smith MF.: Intraoperative 5-fluorouracil for filtration surgery in the rabbit. *Invest. Ophthalmol. Vis. Sci.* 1993; 34, (12): 3313-3319.
7. Roth, S.M., Spaeth, G.L., Starita, R.J., Birbillis, E.M. and Steinmann, W.C.: Effects of postoperative corticosteroids on trabeculectomy and the clinical course of glaucoma. The five-year follow up study. *Ophthalmic Surg.* 1991; 22: 724-729.
8. Weinreb, R.N.: Adjusting the dose of 5-fluorouracil after filtration surgery to minimize side effects. *Ophthalmology.* 1987; 94: 564-670.
9. Finkelstein, I., Trope, G.E., Heathcote, J.G., Rootman, D.S., Spero, L. and Manon, I.A.: Further evaluation of collagen shields as a delivery system for 5-fluorouracil in glaucoma filtration surgery: *Can. J. Ophthalmol.* 1991; 26: 129-132.
10. Charles, J.B., Ganthier, R.J. and Wilson, M.R.: Use of bioerodible polymers impregnated with mytomycine in glaucoma filtration surgery in rabbits. *Ophthalmology.* 1991; 98: 503-508.
11. Khaw, P.T., Doyle, J.W., Sherwood, M.B., Grierson, I., Schultz, G. and McGorray, S.: Prolonged localized tissue effects from 5-minute exposures to fluorouracil and mytomycine C. *Arch. Ophthalmol.* 1993; 111, (2): 263-267.
12. Jampel, H.D., Thibault, D., Leong, K.M., Uppal, P. and Quingley, H.A.: Glaucoma Filtration surgery in nonhuman primates using taxol and etoposide in polyanhydride carriers. *Invest. Ophthalmol. Vis. Sci.* 1993; 34, (11): 3076-3083.
13. Stainhorst, U.H., Chen, E.P., Freedman, S.F., Macheimer, R., and Hatchell, D.L.: Growth inhibition of human Tenon's capsule fibroblasts and rabbit dermal fibroblasts with non-carcinogenic N-alkylated anthracyclines. *Graefes Arch. Clin. Exp. Ophthalmol.* 1994; 232, (6): 347-354.
14. Kitazawa, Y., Kawase, K., Matsushita, H. and Minobe, M.: Trabeculectomy with mytomycine. A comparative study with fluorouracil. *Arch. Ophthalmol.* 1991; 109: 1693-1698.
15. Kitazawa, Y., Taniguchi, T., Nakano, Y., Shirato, S. and Yamamoto, S.: 5-Fluorouracil for trabeculectomy in glaucoma. *Graefes Arch. Clin. Exp. Ophthalmol.* 1987; 225: 403-405.
16. Buffenn, A.N., Seatre, S.B., Higginbotham, E.J., Musch, D.C. and Kincaid, M.C.: Transconjunctival mytomycine-C as an adjunct to conventional glaucoma. *J. Glaucoma.* 1997; 6, (5): 314-318.
17. Iliev, M.E., Zypen, E., Frankhauser, F. and England, C.: Transconjunctival application of mytomycine-C in combination with laser sclerotomy ab interno: A long-term morphological study of the postoperative healing process. *Exp. Eye Res.* 1997; 64, (6): 1031-1026.
18. Hyung, S.M., Kim, D.M., Hong, C., Lee, J. and Young, D.H.: Effects of postoperative mytomycine-C on glaucoma filtration surgery in rabbits treated preoperatively with antiglaucoma medications. *Ophthalmic Surg.* 1994; 25, (10): 704-714.
19. Bair, J.S. and Chen, C.W.: Trabeculectomy with multiple application of mytomycine-C in monkeys with experimental glaucoma. *J. Pharmacol. Ther.* 1997; 13, (2): 115-128.
20. Prata, J.A.J., Seah, S.K.L., Minckler, D.S., Baerveldt, G., Lee, P.P., and Heuer, D.K.: Postoperative complications and short-term outcome after 5-fluorouracil or mytomycine-C trabeculectomy. *J. Glaucoma* 1995; 4: 25-31.
21. Glover, T.L., Nasisse, M.P. and Davidson, M.G.: Effects of topically applied mytomycine-C on intraocular pressure, facility of outflow, and fibrosis after glaucoma filtration surgery in clinically normal dogs. *Am. J. Vet. Res.* 1995; 56, (7): 936-940.
22. Mietz, H., Niesen, U. and Kriegelstein, G.K.: The effect of preservatives and antiglaucomatous medications on the histopathology of the conjunctiva. *Graefes Arch. Clin. Exp. Ophthalmol.* 1994; 232, (9): 561-565.
23. Gressel, M.G., Parrish, R.K. and Folberg, R.: 5-Fluorouracil and glaucoma filtering surgery: I. An animal model. *Ophthalmology.* 1984; 91: 378-383.
24. Miller, M.H., Grierson, I., Unger, W.I. and Hitchings, R.A.: Wound healing in an animal model of glaucoma fistulizing surgery in the rabbit. *Ophthalmology Surg.* 1989; 10: 350-357.
25. Yamamoto, T., Varani, J., Kaz Soong, H and Lichter P.R.: Effect of 5-fluorouracil and mytomycin-C on cultured rabbit subconjunctival fibroblasts. *Ophthalmology.* 1990; 97: 1204-1210.
26. Gilger, B.C., Whitley, D., McLaughlin, S.A., Wright, J.C. and Drane, W.: Canine corneal thickness measured by ultrasonic pachymetry. *Am. J. Vet. Res.* 1991; 52, (10): 1570-1572.

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