Transconjunctival penetration of mitomycin C

T Velpandian, MD; Ramanjit Sihota, MD, FRCO; Ankur Sinha, MD; Viney Gupta, MD

Aims: The study was performed to estimate transconjunctival penetration of mitomycin C (MMC) to Tenon's tissue following application over the intact conjunctiva before routine trabeculectomy.

Settings and Design: Institution-based case series.

Materials and Methods: In 41 eyes of 41 patients, MMC (0.4 mg/ml for 3 min) was applied over the intact conjunctiva before beginning trabeculectomy. Tenon's capsule directly beneath the site of application was excised during trabeculectomy and was homogenized, centrifuged and MMC concentrations were analyzed using high-performance liquid chromatography (HPLC).

Statistical Analysis Used: Statistical analysis was performed using STATA 8.0 version software (STATA Corporation, Houston, TX, USA). In this study, P-values less than 0.05 were considered as statistically significant.

Results: The average weight of the sample of Tenon's tissue excised was 5.51 ± 4.42 mg (range: 0.9-17.1) and the average estimated MMC concentration found to be present in Tenon's tissue using HPLC was 18.67 ± 32.36 × 10⁻⁶ moles/kg of the tissue (range: 0.38-197.05 × 10⁻⁶). In 36 of the 41 patients (87.80%), the MMC concentration reached above 2 × 10⁻⁶ moles/kg of the tissue concentration required to inhibit human conjunctival fibroblasts.

Conclusions: Mitomycin C does permeate into the subconjunctival tissue after supraconjunctival application for 3 min. Application of MMC over the conjunctiva may be a useful alternative to subconjunctival or scleral application during routine trabeculectomy and as an adjunct for failing blebs.

Key words: High-performance liquid chromatography estimation, mitomycin C, supraconjunctival mitomycin, topical application

Indian J Ophthalmol 2008;56:197-201

Cytostatic substances, such as mitomycin C (MMC), have been used intraoperatively as an adjunct in trabeculectomies. The anti-proliferative agents (MMC and 5-florouracil) have improved the outcome of glaucoma-filtering surgeries.1-6 This may be either due to the cytostatic effects manifesting as delayed wound healing resulting in prolonged enhanced filtration or possibly a direct toxic effect on the epithelium of ciliary body or both.4,7

Factors that influence the anti-fibroblastic effects of MMC are the concentration used, area of application, duration of application and material of sponge used.8 The best mode in terms of concentration, duration, place, and number of applications is still not known9 and rationale behind single intraoperative application has also been questioned.10 There have been reports of topical application of MMC after trabeculectomy for post-operative pharmacological modulation.4,6,11 These reports have shown MMC application in the form of topical drops6 and surface application11,6 to be an effective alternative to single intraoperative application. However, there has been no study in the literature estimating the concentration of MMC in Tenon's tissue after surface application of the drug over the intact conjunctiva. The aim of the study was to estimate the concentration of MMC in Tenon's tissue after single topical application of the MMC.

Materials and Methods

After taking clearance from the Institutional Review Board, this pilot study was conducted. The study included patients who were scheduled for a standard trabeculectomy from February 2005 to December 2005. The study was carried out within the tenets of the Declaration of Helsinki. Only eyes of patients not achieving target intraocular pressure (IOP) on maximum tolerable medical therapy (at least three topical medications, which included 0.5% timolol, 0.15% brimonidine, 2% pilocarpine, 0.005% latanoprost or 0.03% bimatoprost along with oral acetazolamide and/or syrup glycerol and intravenous mannitol) were included in this study. The patients with history of prior glaucoma surgery, conjunctival congestion were excluded from the study. After informed consent (see Appendix) and explaining the procedure in detail, a peribulbar block from (6 ml of 2% xylocaine and 4 ml of 0.5% sensoricaine) was given through the skin. All the trabeculectomies were...
performed by two surgeons (R.S. and V.G) using a limbal-based conjunctival flap as described by Cairns. Whole dry week cell sponge (Supersoak sponges; Madhu Instruments, New Delhi, India) were soaked (till it maximally absorbed MMC) with freshly prepared 0.4 mg/ml of MMC and these were then placed directly over the intact bulbar conjunctiva at the proposed site of trabeculectomy for 3 min. The drug was thoroughly washed out of the palpebral sac with 10 ml of the Ringer lactate solution. A superior rectus bridle suture was then passed and 8-10 mm high limbus-based flap was raised. A small piece of Tenon’s tissue was excised just below the area of application and high-performance liquid chromatography (HPLC) estimation of the MMC in sample was done using the procedure described below. The samples were coded and subjected for MMC estimation in the department of ocular pharmacology.

Estimation of MMC from the tissue samples was performed as per the method reported earlier with minor modification. Briefly, the tissues were weighed immediately along with the pre-weighed microcentrifuge tubes upon their arrival. Methanol (HPLC grade) was added at the volume of 200 µl and preserved at −70°C till analysis. On the day of analysis, the samples were homogenized in cold condition using hand-held microcentrifuge tube homogenizer to avoid the evaporation of methanol. Samples were then centrifuged at 5000 × g for 10 min. The supernatant underwent HPLC quantification, thermo surveyor HPLC system was used. The mobile phase containing phosphate buffer (pH6.5) and acetonitrile in the ratio of 8:2 v/v was used. It was pumped at the flow rate of 1 ml/min and the analytical separation was achieved by C8-Kromosil (250 × 4.6 mm, 5 µ) column (Flexit Jour Laboratories Pvt Ltd, Pune, Maharashtra, India). The UV detection was achieved at 365 nm and peak purity was performed over a wavelength range of 200-400 nm using the custom-made library of photodiode array option [Fig. 1].

Under the above conditions, MMC was eluted at the retention time of 6.52 min. The limit of detection of MMC using the above method is 0.427 × 10⁻⁴ moles/kg of tissue. The weight of the tissue samples varied from 0.9 to 17.1 mg with a mean of 5.5 ± 4.42 mg (median 4.2).

Mean concentration and the range of concentrations obtained from the tissues sent were evaluated. The concentration of MMC obtained from the tissue was correlated with the weight of the tissue and the age of the patients. Statistical analysis was performed using stata 8.0 version software (STATA Corporation). In this study, P-values less than 0.05 were considered as statistically significant.

Results

A total of 41 eyes of 41 patients were included in the study. Of the 41 patients, there were 28 males and 13 females. The mean age of the patients was 45.63 ± 18.27 years (range: 3-77 years). The mean pre-operative IOP recorded was 26.61 ± 3.52 mmHg (on maximum tolerable medical therapy). Of the 41 patients, 22 were diagnosed to have chronic primary angle closure glaucoma, nine had primary open angle glaucoma and 10 had other forms of glaucoma [congenital (1), angle recession (1), Axenfeld-Reigers anomaly (2), neovascular glaucoma without significant conjunctival hyperemia and inflammation controlled on steroids (2), secondary glaucoma associated with adherent leukoma (1), operated pars plana vitrectomy (1), Sturge-Weber syndrome (1) and aphakia (1)].

The average weight of the sample of Tenon’s tissue excised was 5.51 ± 4.42 mg (median 4.2 mg with a range 0.9-17.1) and the average estimated MMC concentration was 18.67 ± 3.52 mg/kg (range: 0.38-197.05 × 10⁻⁴). In 36 of the 41 patients (87.80%), the MMC concentration reached above 2 × 10⁻⁴ moles/kg of the tissue (concentration required to inhibit human conjunctival fibroblasts). On statistical analysis, the correlation between the weight of the sample and the MMC concentration (P = 0.2, r = 0.18) and the age of the patient and the MMC concentration detected (P = 0.3, r = 0.16) in the sample was not found to be statistically significant. The results have been summarized in Table 1 and Figs. 2 and 3.

Of the 41 eyes included in the study, shallow anterior chamber (AC) was noted in five cases. Single procedure of AC reformation (under sterile conditions, sterile air was injected in the AC with the help of 26-G needle under topical anesthesia with 0.5% proparacaine) was sufficient to manage four of the five cases with shallow AC. In one case, after two procedures of AC reformation, bleb revision was done to manage shallow AC. In no case was bleb leak, thin bleb, persistent hypotony, choroidal detachment, hypotonic maculopathy or corneal epithelial toxicity noticed in the first post-operative month.

Table 1: The weight (average ± SD) of Tenon’s tissue obtained and concentration (average ± SD) of mitomycin C (MMC) estimated

| Age of patients | No. of patients | Average age (years) | Average weight of Tenon’s tissue taken (mg) | Average concentration of MMC detected (10⁻⁴ moles/kg of tissue) |
|-----------------|----------------|---------------------|-------------------------------------------|----------------------------------------------------------|
| <20 years       | 6              | 14.5 ± 5.82         | 8.32 ± 5.78                               | 15.8 ± 19.49                                             |
| 20-40 years     | 9              | 32.11 ± 7.8         | 5.21 ± 5.18                               | 27.98 ± 63.85                                            |
| 41-60 years     | 18             | 53.44 ± 5.09        | 5.30 ± 4.28                               | 18.93 ± 17.64                                            |
| >60 years       | 8              | 66.62 ± 4.93        | 4.22 ± 1.97                               | 8.06 ± 7.20                                              |
been fraught with problems.6 These experiments further reveal that after 1 min application of 0.2 mg/ml of MMC, the maximum concentrations on the outer side of the sclera may reach 0.01-2 \( \mu \)g/ml depending upon the duration of exposure.19 Jampel has shown that MMC when applied in the concentration of 0.4 mg/ml for a period of 5 min almost totally stops the proliferation of cultured human fibroblasts (90% inhibition).16 Morphologic proof of toxic effects of MMC on non-pigmented epithelium of ciliary body following episcleral application7,19 suggest a role of ciliary hypo-secretion as a cause of persistent hypotony.

The present study conclusively demonstrated that MMC penetrates the conjunctival epithelium and is found in a mean concentration of 18.67 ± 32.36 \( \times 10^{-6} \) moles/kg of the tissue (range: 0.38-197.05 \( \times 10^{-6} \)) in Tenon's tissue. It has been experimentally shown (in vitro) that an MMC concentration of 2 \( \times 10^{-5} \) \( \mu \) (moles/liter) is required to inhibit the proliferation of fibroblasts. In our study, concentration higher than the above mentioned was estimated in 36 of the 41 patients (87.80%) in whom MMC was applied over the intact conjunctiva. Furthermore, supracconjunctival application may reduce the amount of drug diffusion through the sclera as the drug is applied over the conjunctiva and there are barriers of conjunctiva and Tenon's capsule in addition to sclera, thereby reducing the chances of ciliary epithelium toxicity manifesting as prolonged hypotony and other complications. The concentration is represented in moles/kg of tissue since estimation of water content in such a small quantity of human tissue was not possible for the molar calculation for each and every sample.

Post-operative use of MMC in the form of drops (0.3 mg/ml) has been shown to be effective in terms of halting and reversing early subconjunctival fibrosis.6 Corneal epithelial toxicity, however, has been seen with the use of topical drops.

In a study by Mietz et al., post-operative surface application versus intraoperative application of MMC was compared.11 It was found that the hypotony as a transient phenomenon occurred in 36% cases in post-operative application group as compared to 77% cases in the intraoperative application group. The mean IOP at final visit was 16.0 ± 2.7 (9-20) in post-operative application group and 12.5 ± 7.1 (4-32) in the intraoperative application group. None of the cases in the post-operative group reported ocular discomfort or corneal erosions. In our study, transient hypotony requiring AC reformation or bleb revision was noted in 5 of the 41 (12.20%) cases, no cases of bleb leak, thin bleb, persistent hypotony, choroidal detachment, hypotonic maculopathy or corneal epithelial toxicity was noticed in the immediate post-operative period.

It is well proven that anti-metabolites, when used with needle revision enhance the success of failing blebs.20 During these procedures subconjunctival injection of 5 FU are given in the bleb area. On noticing the early signs of bleb failures such as bleb injection, bleb vascularity or large ropy vessels20 over the bleb area, MMC may be applied supraconjuunctivally. Our study shows that MMC freely penetrates the conjunctiva and would probably inhibit the proliferation of fibroblasts underneath. Supraconjunctival

Discussion

Mitomycin C is an antibiotic, alkylating agent, which acts by binding to DNA and inhibits cell mitosis and interferes with RNA transcription and protein synthesis7,15 and is found to inhibit proliferation of cultured human Tenon's fibroblasts after a brief exposure of 1-5 min.6

Mitomycin C is now used intraoperatively in the glaucomatous eyes having a high risk of surgical failure. However, in some eyes, the need for an anti-fibrotic agent is felt only after surgery when subconjunctival scarring or encystment of the bleb is seen. MMC drops have been applied but have been fraught with problems.6

Wound healing is a complex process and there are many inter-individual variations apart from variations among races. Even in the present study, there was a wide variation of the amount of MMC detected in different tissue samples for the same amount of concentration applied topically; this could be accounted for by the differences in conjunctival and Tenon's thickness in different individuals.

Pharmacokinetics of MMC has been studied and it has been shown experimentally on human donor eyes that MMC penetrates the sclera easily and irrigation reduces the concentration only in the superficial layers.17 It is further shown that during trabeculectomy in human eyes, the diffusion of MMC to the deeper layers continues even after the end of exposure to the soaked sponge.18 These experiments further reveal that after 1 min application of 0.2 mg/ml of MMC, the maximum concentrations on the inner side of the sclera may reach 0.01-2 \( \mu \)g/ml depending upon the duration of exposure.19 Jampel has shown that MMC when applied in the concentration of 0.4 mg/ml for a period of 5 min almost totally stops the proliferation of cultured human fibroblasts (90% inhibition).16 Morphologic proof of toxic effects of MMC on non-pigmented epithelium of ciliary body following episcleral application7,19 suggest a role of ciliary hypo-secretion as a cause of persistent hypotony.
Appendix: Consent Form for Trabeculectomy and Study

Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences

Glaucoma Consent Form

About trabeculectomy
My doctor has informed me that the operation is necessary to help control the pressure in my eye(s). If the pressure remains too high it may result in blindness in my eye. There is no guarantee that the operation will control the pressure and other procedures, including more operations, may be essential.

Glaucoma operations may be followed by complications. Among these complications are: (i) it is common to have decreased vision for about two months, (ii) a cataract may develop and this may have to be removed at some future date, and (iii) sometimes the pressure in the eye becomes too low or too high and this may necessitate other treatments or operation.

It is not unusual that I may have to continue treating the eye with drops or ointments or medications taken by mouth or the doctor may have to give injections around the eye. There are also risks that accompany anesthesia.

Not every conceivable complication can be covered in this form.

In spite of the risks noted above, I understand that there is more risk if I do not have the operation, than that if I do.

About the study
I have also been informed that a drug called mitomycin C is commonly used to increase the success of the above-mentioned surgery; however, its usage may lead to an increase in complications described earlier. In addition, it is explained to me that rather than using the drug during the surgery, it would be used before the start of surgery. This type of usage has been used in the past, after the surgery. Furthermore, a small piece of tissue layer (Tenon's) beneath the conjunctiva would be taken to estimate the amount of drug in it. This may affect the outcome of the surgery in an unpredictable manner.

Knowing all this, I willingly give consent to participate in the study and undergo surgery.

Occasionally, a different, unsuspected condition may arise at the time of surgery and I authorize my surgeon to do what he/she seems necessary. I understand that no warranty or guarantee has been made to me regarding the result, cure or safety.

I also understand that during the course of the proposed procedure(s), unforeseen conditions may be revealed requiring the performance of additional procedures and I authorize such procedures to perform.

I have read and understand the consent form, my questions have been answered and I authorize my surgeon to proceed with the operation.

Patient (or person authorized to sign for patient)
Date
Witness

application of MMC may provide a less invasive alternative to subconjunctival application, especially when used along with bleb revision. We believe that in cases showing early signs of failing bleb, one can opt for post-operative surface application of MMC.

In our study, there was large variation in the amount of tissue harvested and the concentration of MMC achieved in Tenon's tissue; however, in most of the cases (80.80%), the MMC concentration reached above 2 x 10^-6 moles/kg of the tissue. Despite the limitations of the study, in that there was no standardization of the amount of tissue excised for analysis and only a single concentration of 0.4 mg/ml for a fixed duration of time (3 min) was studied, the quantitative information regarding the transconjunctival penetration of supraconjunctival MMC is clear.

This pilot study conclusively proves that significant concentrations of MMC can be achieved by a single supraconjunctival application of 0.4 mg/ml. This mode of application may be a less invasive and theoretically a safer alternative to subconjunctival application of MMC, especially for revival of a failing bleb and provide an effective penetration of anti-metabolite at the required site.

References
1. Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. Ophthalmology 1991;98:317-21.
2. The Fluorouracil Filtering Surgery Study Group. Three year follow-up of the fluorouracil filtering surgery study. Am J Ophthalmol 1993;115:82-92.
3. Kupin TH, Juzych MS, Shin DH, Khatana AK, Olivier MM. Adjunctive mitomycin C in primary trabeculectomy in phakic eyes. Am J Ophthalmol 1995;119:30-9.
4. Mietz H, Jacobs PC, Kriegsstein GK. Intraoperative episcleral versus postoperative topical application of mitomycin-C for trabeculectomies. Ophthalmology 2002;109:1343-9.
5. WuDunn D, Cantor LB, Palanca-Capistro AM, Hoop J, Alvi NP, Finley C, et al. A prospective randomized trial comparing intraoperative 5-fluorouracil vs mitomycin C in primary trabeculectomy. Am J Ophthalmol 2002;134:521-8.
6. Sihota R, Sood NN, Agarwal HC. Mitomycin C drops in the postoperative pharmacological modulation of trabeculectomies. Ann Ophthalmol 1997;26:362-6.
7. Nyuys RM, Felten PC, Pels E, Langerhorst CT, Geijssen HC, Grossniklaus HE, et al. Histopathologic effects of mitomycin C after trabeculectomy in human glaucomatous eyes with persistent hypotony. Am J Ophthalmol 1994;118:225-37.
8. Kim YY, Sexton RM, Shin DH, Kim C, Ginde SA, Ren J, et al. Outcomes of primary phakic trabeculectomies without versus with 0.5- to 1-minute versus 3- to 5-minute mitomycin C. Am J Ophthalmol 1998;126:735-62.
9. Esser J, Esser P, Mietz H, Hueber A, Kociok N, Schraemeyer U, et al. Multidrug resistance-associated proteins in glaucoma surgery. Graefes Arch Clin Exp Ophthalmol 2000;238:727-32.
10. Khaw PT, Sherwood MB, MacKay LD, Rossi MJ, Schultz G. Five minutes treatments with fluorouracil, fluorouridine and mitomycin have long term effects on human Tenon's capsule fibroblasts. Arch Ophthalmol 1992;110:1150-4.
11. Mietz H, Jacobs PC, Kriegsstein GK. Postoperative application of Mitomycin for Trabeculectomy. Arch Ophthalmol 2000;118:1341-8.
12. Cairns JE. Trabeculectomy: Preliminary report of a new method. Am J Ophthalmol 1968:66:673-9.
13. Velpandian T, Saluja V, Ravi AK, Kumari SS, Mathur R, Ranjan N, et al. Evaluation of the stability of extemporaneously prepared ophthalmic formulation of mitomycin C. J Ocul Pharmacol Ther 2005;21:217-22.

14. Lee DA, Lee TC, Corres AE, Kirada S. Effects of mifluramycin, mitomycin, daunorubicin and bleomycin on human subconjunctival fibroblast attachment and proliferation. Invest Ophthalmol Vis Sci 1990;31:2136-44.

15. Zacharia PT, Deppermann SR, Schuman JS. Ocular hypotony after trabeculectomy with Mitomycin-C. Am J Ophthalmol 1993;116:314-26.

16. Jampel HD. Effect of brief exposure to mitomycin C on viability and proliferation of cultured human Tenon's fibroblasts. Ophthalmology 1992;99:1471-6.

17. Vass C, Georgopoulos M, el Menyawi I, Radda S, Nimmerrichter P. Intrasceral concentration vs depth profile of mitomycin-C after episcleral application: Impact of applied concentration and volume of mitomycin-C solution. Exp Eye Res 2000;70:571-5.

18. Georgopoulos M, Vass C, El Menyawi I, Radda S, Graninger W, Menapace R. In vitro diffusion of mitomycin-C into human sclera after episcleral application: Impact of diffusion time. Exp Eye Res 2000;71:453-7.

19. Schraermeyer U, Diestelhorst M, Bieker A, Theisohn M, Mietz H, Ustundag C, et al. Morphologic proof of the toxicity of mitomycin C on the ciliary body in relation to different application methods. Graefes Arch Clin Exp Ophthalmol 1999;237:593-600.

20. Broadway DC, Bloom PA, Bunce C, Thiagarajan M, Khaw PT. Needle revision of failing and failed trabeculectomy blebs with adjunctive 5-fluorouracil: Survival analysis. Ophthalmology 2004;111:665-73.

21. Shingleton BJ. Management of the failing glaucoma filter. Ophthalmic Surg Lasers 1996;27:445-51.

Source of Support: Nil, Conflict of Interest: None declared.