ORIGINAL ARTICLE

The effect of 0.02% Mitomycin-C drop in prevention of epithelial ingrowth in corneal stroma of rabbit as an animal experimental model

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Abstract

Background: Mitomycin–C (MM-C) is an alkylating anti-tumor antibiotic, which interrupts DNA replication and inhibits routine synthesis and cell mitosis. This study was undertaken to evaluate the effect of MM-C on the epithelial ingrowth which is a sight threatening complication after corneal surgeries.

Methods: This study was conducted on 50 corneas of 25 Dutch Albino rabbits. Lamellar keratectomy was performed and after the application of MM-C 0.02 % or balanced salt solution (control group), the epithelial cells were implanted on the stromal bed. After sixty days, light microscopic exam was performed on the excised corneas.

Results: Three eyes in the MM-C group and no eye in the control group showed epithelial cell growth under the corneal flap.

Conclusion: In this study, application of MM-C 0.02% drop at the time of operation had no preventive role in the corneal epithelial cell proliferation under rabbit's corneal flap. So, implantation of the epithelial cells under the corneal flap is not the sole causative factor of epithelial ingrowth in the stroma of the rabbit's cornea.

Keywords: Mitomycin –C; Epithelial cell; Cornea; Balanced salt solution

Introduction

Mitomycin–C (MM-C) is an alkylating anti-tumor antibiotic, which was isolated for the first time from *Streptomyces caespitosus* by Hata in 1956.[1] MM-C interrupts DNA replication and inhibits routine synthesis and cell mitosis. It is active against all cells regardless of the phase of the cell cycle, but it has the greatest anti-proliferative effect on the cells with the highest rate of mitosis.[1] MM-C has different applications in ophthalmology. It has been used to prevent pterygium recurrence after surgery since early 1960s,[2-8] and to increase the chance of success of glaucoma filtering surgery.[9-12] It has been used as an alternate treatment for conjunctival intraepithelial neoplasia (CIN),[13-16] and as a pharmacological method to modulate healing response, decrease fibrosis, and improve the predictability and long term stability of photorefractive keratectomy (PRK).[17-18] Animal studies have shown reduced keratocyte population and corneal haze in the eyes that had undergone PRK by Excimer laser followed by a single topical application of a low dose of MMC.[19-20]

One major complication of LASIK surgery is epithelial ingrowth under the corneal flap.[21] It can occur when a membrane is connected to the flap junction, due to interface junctional problem, or when isolated islands are separated from the junction area due to epithelial cell implantation. Epithelial ingrowth can cause visual disturbance and flap complication, and irrigation of the stromal bed might not improve the situation and even cause further complications. We had experimentally tried 0.02% MM-C drop in

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Received: May 12, 2007 Accepted: November 18, 2007

two patients with recurrent epithelial ingrowth under corneal flap after LASIK reoperation surgery. The ingrowths were isolated epithelial islands without connection to the flap junction and any clinical junctional problem. Before MM-C trial, irrigation of stromal bed had been performed twice but again epithelial ingrowth recurred.

The drug was given four times a day for one week. After 7 days, another one-week course of treatment was started. After about one month, the epithelial cell population decreased in one case and symptoms such as deteriorated visual acuity and photophobia decreased in both cases. So, we designed this study in rabbits to evaluate the possible preventive effect of MM-C on epithelial cell growth implanted under the corneal flap.

Materials and Methods

In Laboratory Animal Center of Shiraz University of Medical Sciences, 50 eyes of 25 Dutch Albino rabbits of 10-12 months and 2-2 ½ kg of weight were operated under general anesthesia (ketamine 44 mg/kg/IM+ xylazine 8 mg/kg IM), using operating microscope. These normal eyes were used according to the protocol approved by the Ethics Committee of Shiraz University of Medical Sciences. After preparing with 10% Betadine and drape by the eye sheath, a stromal corneal flap with temporal hinge was prepared bilaterally by a crescent knife (Alcon Company). Randomly, on one stromal bed balanced salt solution (BSS) drop (control group) and on the other stroma MM-C 0.02 % drop was applied. The surgeon was blind to the application of both drops. After a minute, the cornea was irrigated by 10 cc normal saline solution; abraded corneal epithelial cells from the same eye were embedded under the flap which was then replaced back. Temporary suture tarsorrhaphy was performed for 5 days and after 60 days; two ophthalmic pathologists evaluated the excised corneas (H & E), using light microscopy, looking for any evidence of epithelial cells under the flap.

Results

Among 50 rabbit eyes, 2 corneas (one from each group) were traumatized accidentally and so they were excluded from this study. Of the remaining 48 eyes, interface epithelial cell growth was observed in 3 corneas in the MM-C applied group (Table 1). One of the MM-C applied cases showed decreased keratocyte count and another one keratitis (PMN and lymphocyte cell infiltration). Other findings such as interface scaring, keloid formation, stromal interface vascularization, stromal and flap edema, epithelial hyperplasia over incision and the flap occurred with the same frequency among the MM- C and non-MM-C groups (Table 1).

Stromal scaring was less in the MM-C group. Eosinophilic staining in the interface was present in two and epithelial thinning over the flap in three of the non-MM-C group (Table 1). Twelve corneas showed no pathologic findings. Of them, nine were from MM-C and three from non-MM-C groups (Table 1). None of the corneas showed any endothelial change.

Table 1: Histopathologic findings

Pathology features	Total number of corneas involved	Percentage	NO. of corneas in MM-C group	NO. of corneas in non MM-C group
Epithelial hyperplasia over the flap incision	14	29.16	7/14	7/14
Stroma and flap edema	9	18.75	4/9	5/9
Stromal and interface vascularization	4	8.32	2/4	2/4
Keloid formation	4	8.32	2/4	2/4
Interface scaring	2	4.16	1/2	1/2
Decreased keratocyte	1	2.08	1/1	0/1
Keratitis (PMN)	1	2.08	1/1	0/0
Flap fibrosis	7	14.56	2/7	5/7
Stromal scaring	2	4.16	0/2	2/2
Epithelial thinning over flap	3	6.24	0/3	3/3
Eosinophilic staining in the interface	2	4.16	0/2	2/2
Epithelial cells in the interface	3	6.24	3/3	0/3
No pathologic finding	12	24.96	9/12	3/12

Histopathologic findings in rabbit's cornea after MM-C or BSS application

Discussion

Based on our clinical trial to treat epithelial ingrowth, we designed this study in rabbits to evaluate the possible preventive effect of MM-C on epithelial cell growth implanted under the corneal flap. Actually, we do not have any explanation for the clinical response of our patients to MM-C. Probably there was some fibrous tissue component associated with epithelial ingrowth and this was the reason that MM-C proved to be effective. The research by Dr Ando showed that MM-C can inhibit epithelial wound healing in the rabbit cornea.[22] It has been shown that 0.02% MM-C can decrease the corneal haze in patients that have developed sub-epithelial fibrosis after PRK.[17,18]

Two months after implanting the epithelial cells in the stroma of rabbit cornea, the light microscopic histopathological study showed epithelial cells in the interface of three corneas, all belonging to the MM-C group. This means that 0.02% MMC, probably, has either no preventive effect on the epithelial growth or causes the flap junctional defect which promotes epithelial ingrowth. Along with search for epithelial cell growth, other findings were also obtained. Epithelial hyperplasia on the flap incision site, stromal flap edema, stromal and interface vascularization, keloid formation, and interface scaring occurred equally in both MM-C and non-MM-C groups. Injured epithelial cells, sustained in the LASIK flap and during LASIK or PRK surgery, release cytokines such as IL-1 and then Fas/Fas ligand that causes keratocyte apoptosis.[23-25] In this study, only one cornea in the MM-C group showed decreased keratocyte and this was after the epithelium had healed (no more stimuli for keratocyte apoptosis). After regional keratocyte apoptosis, keratocyte proliferation and myofibroblast transformation occur, leading to scaring. This fibrosis can be controlled medically by corticosteroid and MM-C.[26-30] In this study, less stromal fibrosis was found in the MM-C group, too. After refractive surgery, epithelial hyperplasia can occur.[31-37] This change takes place under the effect of mediators such as transforming growth factor (TGF) and epithelial growth factor (EGF), and can lead to regression. In this study, 14 corneas, 7 in each group, showed epithelial hyperplasia. Additionally, epithelial thinning occurred in only one of non-MM-C group (control group). In MM-C group, there was no change in the epithelial thickness or there was epithelial hyperplasia. In other words, MM-C had no preventive effect on the surface epithelial cell growth. Finally, 12 corneas (25%) had no pathological change, among which 9 belonged to MM-C group. None of the corneas showed any endothelial change.

The results revealed that 0.02% MMC use could not prevent epithelial ingrowth under the corneal flap of the rabbit eyes. In addition, not every epithelial cell implanted in the corneal stroma can survive or grow.

Acknowledgement

The authors would like to thank Dr. D. Mehrabani at Center for Development of Clinical Studies of Nemazee Hospital for editorial assistance.

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