Published in final edited form as:
J Refract Surg. 2009 October ; 25(10): 894–897. doi:10.3928/1081597X-20090617-10.

Impact on the Corneal Endothelium of Mitomycin C During Photorefractive Keratectomy

Danny S. Roh, BS and **James L. Funderburgh, PhD**
Department of Ophthalmology, UPMC Eye and Ear Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pa

Abstract

PURPOSE—This brief review examines both basic science and clinical studies to evaluate the potential impact on the health of the corneal endothelium of mitomycin C (MMC) usage during photorefractive keratectomy (PRK).

METHODS—The mechanism of action and consequences of MMC are reviewed within the context of in vitro, animal, and clinical studies and a hypothesis of how this vital cell layer responds to MMC at both the cellular and clinical levels is formed.

Mitomycin C (MMC) was proposed to be an effective modulator of the wound healing response after corneal stromal ablation as early as 1991.[1] Since then, MMC usage has expanded significantly within the field of refractive surgery, especially during photorefractive keratectomy (PRK), for prevention of recurrence of corneal haze and as prophylaxis to prevent initial haze development.2 Despite the growing popularity of MMC usage during refractive procedures, there has been inconsistency in the conclusions of studies regarding the impact of MMC on the corneal endothelium. Because of the non-regenerative nature of the human corneal endothelium, it is imperative to understand how this vital cell layer responds to potential stressors at both the cellular and clinical levels. This brief review examines both basic science and clinical studies to evaluate the potential impact of MMC usage during PRK on the health of the corneal endothelium.

MECHANISM OF ACTION OF MITOMYCIN C

Mitomycin C is an antibiotic derived from *Streptomyces caespitosus* and is generally classified as a DNA alkylating agent. Cellular toxicity after MMC exposure can occur from MMC-induced insults such as the generation of free radicals or DNA monoadducts; however, the most significant effects are due to the accumulation of covalent DNA interstrand cross-links.[3] A brief explanation of the mechanism of action of MMC is essential to understand the potential impact of MMC used during PRK on the corneal endothelium.

Correspondence: James L. Funderburgh, PhD, 203 Lothrop St, UPMC Eye and Ear Institute, Rm 1011, Pittsburgh, PA 15213. Tel: 412.647.3853; Fax: 412.647.5880; jlfunder@pitt.edu.

AUTHOR CONTRIBUTIONS

Study concept and design (D.S.R., J.L.F.); data collection (D.S.R.); interpretation and analysis of data (D.S.R., J.L.F.); drafting of the manuscript (D.S.R.); critical revision of the manuscript (J.L.F.); obtained funding (J.L.F.); administrative, technical, or material support (J.L.F.); supervision (J.L.F.)

The authors have no financial or proprietary interest in the materials presented herein.

MMC Activation by Enzymatic Reduction

Mitomycin C by itself is non-reactive with DNA and after entering the cell must undergo bioreductive enzymatic activation. Numerous intracellular enzymes can activate MMC such as NAD(P)H:quinone oxidoreductase 1 (NQO1), which is present throughout all layers of the cornea.[4] Intracellular bioreductive activation results in the production of an unstable intermediate with high alkylating activity and formation of a covalent monoadduct with DNA. Subsequently, this activated MMC will form a second alkylating center, which completes the DNA cross-link. The result is a covalent bond between two complementary strands of DNA, which if not removed, prevents DNA replication and transcription. These MMC–DNA cross-links occur within the minor groove of DNA causing relatively little DNA duplex distortion. The location of adducts is guanine specific and cross-links are located at opposing sequences of 5′-CpG-3′ (CpG).3

Processing and Repair of DNA Interstrand Cross-links

Specific recognition factors of the MMC-induced interstrand cross-link are not well defined, but may occur when DNA replication and/or transcription is blocked within the cell. Importantly, the MMC-induced interstrand cross-link may not be easily repaired in non-replicating mammalian cells because a stalled replication fork typically serves as the signal for initiating DNA repair. Although detailed repair mechanisms in mammalian cells have not been fully elucidated, interstrand cross-link repair is believed to involve a complex combination of nucleotide excision repair, DNA double strand break repair via homologous recombination or non-homologous end joining, and/or translesion DNA synthesis.[5–7] One of the intermediates in interstrand cross-link repair, the DNA doublestrand break, is known to be a potentially lethal lesion. Such breaks can be visualized as γ-H2AX nuclear foci.[8,9]

Consequences of MMC-induced DNA Interstrand Cross-links

Cytotoxicity of MMC-induced interstrand cross-links is most severe in cells undergoing active proliferation, although the details of the responses are specific to the cell type. The presence of a DNA interstrand cross-link during replication induces a stalled replication fork triggering multiple signaling pathways for cell cycle arrest, repair, or apoptosis. Initial responses to MMC-induced interstrand cross-links include a strong activation of p53 and genes downstream.[10, 11] Longer term consequences of such interstrand cross-links include increased apoptosis, cellular senescence, and premature aging.[12]

REVIEW OF BASIC SCIENCE AND ANIMAL STUDIES

The effects of MMC exposure on corneal endothelium have been studied in both in vitro and animal models and have generated informative data about MMC corneal penetration and endothelial toxicity. In three separate studies, the corneal penetration of a single topical application of MMC has been observed during protocols emulating PRK. Using high-performance liquid chromatography (HPLC), Torres et al[13] demonstrated the presence of MMC in hen aqueous humor after a single 0.02% intraoperative application during PRK. Song et al14 also used HPLC to demonstrate the presence of MMC in both cornea and aqueous humor after a single 0.02% MMC application in a rabbit model of epithelial debridement. Our recent study used a bioassay to demonstrate significant MMC concentrations at the depth of the corneal endothelium after a single topical 0.02% application in ex vivo goat corneas.15 It is clear from these studies that refractive surgery procedures such as PRK that involve the mechanical removal of the corneal epithelial barrier, excimer laser stromal ablation, and subsequent MMC treatment, expose the corneal endothelium to biologically substantial levels of MMC. Length of exposure and MMC concentration in the endothelium and aqueous are directly related to the amount of MMC applied to the anterior surface.13,[14]

Surprisingly, despite the widespread use of MMC during refractive surgery, only a few studies have specifically focused on the effects of MMC on the corneal endothelium at the cellular level. Although many more studies describe the keratocyte response to MMC, the response of the corneal endothelium should not be inferred from those results. An early 1991 study by McDermott et al[16] using four pairs of ex vivo human corneas found few changes in mean swelling rate or corneal endothelial ultrastructure after perfusion with 20 μg/mL MMC but found significant irreversible changes at 200 μg/mL. Later in vitro studies have shown dose-dependent toxic effects of MMC on corneal endothelial proliferation.[17,18] Perhaps the most cited study demonstrating MMC toxicity to corneal endothelium was by Chang[19] in 2004, using a rabbit model of mechanical epithelial debridement followed by 0.02% MMC treatments. Over a 2-week period, MMC resulted in dose-dependent apoptosis of the endothelium resulting in increased corneal thickness and decreased corneal clarity.[19] Our recent study[15] demonstrated that even a small and brief dose of MMC significantly alters corneal endothelial DNA, producing significant potential downstream effects. Using MMC concentrations and exposure times that the corneal endothelium is most likely to encounter during intraoperative treatment, we found significant DNA cross-linking and persistent DNA doublestrand breaks in goat corneas ex vivo.[15] The proportion of apoptotic cells significantly increased in MMC-treated cells; however, this number was smaller than the proportion of cells that had DNA doublestrand breaks. Thus, MMC appears to generate living cells with an accumulation of unrepaired DNA damage. Most important, we found that very brief exposures and low concentrations of MMC consistently induced changes in the corneal endothelial monolayer.[15] Thus, it is highly likely that a single topical application of MMC alters corneal endothelial DNA generating downstream effects on cell viability. It remains to be determined what the long-term fate of these DNA cross-links and double strand breaks is and if the same phenomenon occurs in the in vivo setting.

REVIEW OF CLINICAL STUDIES

In contrast to the relatively small number of laboratory studies described above, a larger number of clinical reports have focused on the effects of a single intraoperative dose of MMC during refractive surgery on the corneal endothelium. Whereas a majority of the basic science studies reported significant corneal endothelial toxicity after MMC exposure, the clinical studies have been less conclusive in their evaluation of corneal endothelial effects. Corneal endothelial cell loss has been the major endpoint of these studies carried out by statistical comparisons of pre- and postoperative endothelial central density measurements with occasional use of ultrasound pachymetry.

The initial studies investigating this phenomenon generally reported that MMC treatment during PRK has no measurable effect on corneal endothelial central density. These include a few retrospective[20] and prospective studies.[21,22] Lee et al[20] examined 1011 eyes in a noncomparative retrospective study and determined that endothelial density was not affected by 0.02% MMC treatments ranging from 30 seconds to 2 minutes during PRK after 3, 6, and 16 months. Goldsberry et al[21] found no changes in density or morphology after 1 year in 16 eyes treated with 0.02% MMC for 12 seconds. Diakonis et al[22] followed 15 eyes after PRK with 0.02% MMC for 15 seconds and compared this to epi-LASIK in the fellow eyes. They found a decrease in endothelial density at 1 and 3 months after surgery, however, it was not statistically significant.[22]

In contrast, clinical studies associating MMC use with significant corneal endothelial loss are fewer in number and more recent than those reporting no effect. Two recent studies found significant decreases in endothelial central density after PRK with MMC.[23,24] Nassiri et al, [23] in a nonrandomized, prospective study of 162 eyes, determined that intraoperative 0.02% MMC caused significant corneal endothelial cell loss over a 6-month period that was correlated

with the duration of MMC exposure during PRK. In a prospective study of 18 eyes, Morales et al[24] found that 0.02% MMC exposure of 30 seconds caused significant cell loss at 1 and 3 months.

Although the results from PRK studies do not agree as to a detrimental effect of MMC treatment on corneal endothelial density, the potential for significant MMC toxicity on the corneal endothelium has been well documented in other ocular procedures. Avisar et al[25] found that a 5-minute 0.02% MMC treatment on bare sclera after pterygium resection led to significant postoperative endothelial cell loss up to 3 months postoperatively. A case report by Mohammadpour et al[26] described irreversible focal corneal decompensation after filtering surgery with MMC. Mietz et al[27] reported two cases of bullous keratopathy after trabeculectomy with MMC. Continued MMC treatment in the form of repeated drops after phototherapeutic keratectomy resulted in permanent corneal edema as reported by Pfister.[28] These case reports validate the potential for MMC-induced corneal endothelial damage across various settings.

EXPLANATION OF POSSIBLE DIFFERENCES AND FUTURE RESEARCH

Based on the established mechanism of MMC action, in vitro and animal studies, and reports of endothelial cells loss after MMC in non-PRK treatments, it is apparent that MMC has the intrinsic potential to damage the corneal endothelial DNA under conditions of current clinical use. How can this vulnerability be consistent with the number of clinical reports that describe little or no loss of endothelial central density? Our recent study showed that in goat corneas, the number of cells exhibiting long-term DNA damage by MMC greatly outnumbered the cells undergoing apoptosis in response to clinical concentrations of MMC.[15] If similar DNA cross-linking is occurring in human eyes we might expect, ultimately, to see increased apoptosis in the monolayer of those cells that are unable to tolerate further DNA damage. However, the profoundly quiescent nature of the human endothelium may lead to less cell death in the short-term as a result of these insults. Thus, the corneal endothelium may simply “silently” accumulate this DNA damage without an immediate pathological response. It remains to be determined how the corneal endothelium will eventually respond to such MMC-induced DNA damage over a period of years or decades. Considering unequivocal data demonstrating irreversible DNA damage in endothelium at MMC concentrations now used clinically, it will be important to initiate follow-up studies of treated individuals to identify any potential long-term effects.

Acknowledgments

This work was supported by National Institutes of Health grants EY016415, EY009368, and P30-EY08098; a Research to Prevent Blindness Medical Student Fellowship; and the Eye and Ear Foundation of Pittsburgh. Dr Funderburgh is a Jules and Doris Stein Research to Prevent Blindness Professor.

References

1. Talamo JH, Gollamudi S, Green WR, De La Cruz Z, Filatov V, Stark WJ. Modulation of corneal wound healing after excimer laser keratomileusis using topical mitomycin C and steroids. Arch Ophthalmol 1991;109:1141–1146. [PubMed: 1907822]
2. Lacayo GO III, Majmudar PA. How and when to use mitomycin-C in refractive surgery. Curr Opin Ophthalmol 2005;16:256–259. [PubMed: 16000900]
3. Tomasz M. Mitomycin C: small, fast and deadly (but very selective). Chem Biol 1995;2:575–579. [PubMed: 9383461]
4. Schelonka LP, Siegel D, Wilson MW, Meininger A, Ross D. Immunohistochemical localization of NQO1 in epithelial dysplasia and neoplasia and in donor eyes. Invest Ophthalmol Vis Sci 2000;41:1617–1622. [PubMed: 10845577]

5. McHugh PJ, Spanswick VJ, Hartley JA. Repair of DNA interstrand crosslinks: molecular mechanisms and clinical relevance. Lancet Oncol 2001;2:483–490. [PubMed: 11905724]
6. Noll DM, Mason TM, Miller PS. Formation and repair of interstrand cross-links in DNA. Chem Rev 2006;106:277–301. [PubMed: 16464006]
7. Dronkert ML, Kanaar R. Repair of DNA interstrand cross-links. Mutat Res 2001;486:217–247. [PubMed: 11516927]
8. De Silva IU, McHugh PJ, Clingen PH, Hartley JA. Defining the roles of nucleotide excision repair and recombination in the repair of DNA interstrand cross-links in mammalian cells. Mol Cell Biol 2000;20:7980–7990. [PubMed: 11027268]
9. Niedernhofer LJ, Odijk H, Budzowska M, van Drunen E, Maas A, Theil AF, de Wit J, Jaspers NG, Beverloo HB, Hoeijmakers JH, Kanaar R. The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced doublestrand breaks. Mol Cell Biol 2004;24:5776–5787. [PubMed: 15199134]
10. Abbas T, Olivier M, Lopez J, Houser S, Xiao G, Kumar GS, Tomasz M, Bargonetti J. Differential activation of p53 by the various adducts of mitomycin C. J Biol Chem 2002;277:40513–40519. [PubMed: 12183457]
11. Boamah EK, White DE, Talbott KE, Arva NC, Berman D, Tomasz M, Bargonetti J. Mitomycin-DNA adducts induce p53-dependent and p53-independent cell death pathways. ACS Chem Biol 2007;2:399–407. [PubMed: 17530733]
12. Grillari J, Katinger H, Voglauer R. Contributions of DNA interstrand cross-links to aging of cells and organisms. Nucleic Acids Res 2007;35:7566–7576. [PubMed: 18083760]
13. Torres RM, Merayo-Lloves J, Daya SM, Blanco-Mezquita JT, Espinosa M, Nozal MJ, Bernal JL, Bernal J. Presence of mitomycin-C in the anterior chamber after photorefractive keratectomy. J Cataract Refract Surg 2006;32:67–71. [PubMed: 16516781]
14. Song JS, Kim JH, Yang M, Sul D, Kim HM. Concentrations of mitomycin C in rabbit corneal tissue and aqueous humor after topical administration. Cornea 2006;25:S20–S23. [PubMed: 17001187]
15. Roh DS, Cook AL, Rhee SS, Joshi A, Kowalski R, Dhaliwal DK, Funderburgh JL. DNA cross-linking, doublestrand breaks and apoptosis in corneal endothelial cells after a single exposure to mitomycin-C. Invest Ophthalmol Vis Sci 2008;49:4837–4843. [PubMed: 18658091]
16. McDermott ML, Wang J, Shin DH. Mitomycin and the human corneal endothelium. Arch Ophthalmol 1994;112:533–537. [PubMed: 8155053]
17. Garweg JG, Wegmann-Burns M, Goldblum D. Effects of daunorubicin, mitomycin C, azathioprine and cyclosporin A on human retinal pigmented epithelial, corneal endothelial and conjunctival cell lines. Graefes Arch Clin Exp Ophthalmol 2006;244:382–389. [PubMed: 16091952]
18. Wu KY, Hong SJ, Huang HT, Lin CP, Chen CW. Toxic effects of mitomycin-C on cultured corneal keratocytes and endothelial cells. J Ocul Pharmacol Ther 1999;15:401–411. [PubMed: 10530701]
19. Chang SW. Early corneal edema following topical application of mitomycin-C. J Cataract Refract Surg 2004;30:1742–1750. [PubMed: 15313301]
20. Lee DH, Chung HS, Jeon YC, Boo SD, Yoon YD, Kim JG. Photorefractive keratectomy with intraoperative mitomycin-C application. J Cataract Refract Surg 2005;31:2293–2298. [PubMed: 16473220]
21. Goldsberry DH, Epstein RJ, Majmudar PA, Epstein RH, Dennis RF, Holley G, Edelhauser HF. Effect of mitomycin C on the corneal endothelium when used for corneal subepithelial haze prophylaxis following photorefractive keratectomy. J Refract Surg 2007;23:724–727. [PubMed: 17912945]
22. Diakonis VF, Pallikaris A, Kymionis GD, Markomanolakis MM. Alterations in endothelial cell density after photorefractive keratectomy with adjuvant mitomycin. Am J Ophthalmol 2007;144:99–103. [PubMed: 17509511]
23. Nassiri N, Farahangiz S, Rahnavardi M, Rahmani L. Corneal endothelial cell injury induced by mitomycin-C in photorefractive keratectomy: nonrandomized controlled trial. J Cataract Refract Surg 2008;34:902–908. [PubMed: 18498993]
24. Morales AJ, Zadok D, Mora-Retana R, Martínez-Gama E, Robledo NE, Chayet AS. Intraoperative mitomycin and corneal endothelium after photorefractive keratectomy. Am J Ophthalmol 2006;142:400–404. [PubMed: 16935583]

25. Avisar R, Avisar I, Bahar I, Weinberger D. Effect of mitomycin C in pterygium surgery on corneal endothelium. Cornea 2008;27:559–561. [PubMed: 18520505]

26. Mohammadpour M, Jabbarvand M, Javadi MA. Focal corneal decompensation after filtering surgery with mitomycin C. Cornea 2007;26:1285–1287. [PubMed: 18043196]

27. Mietz H, Roters S, Krieglstein GK. Bullous keratopathy as a complication of trabeculectomy with mitomycin C. Graefes Arch Clin Exp Ophthalmol 2005;243:1284–1287. [PubMed: 15940484]

28. Pfister RR. Permanent corneal edema resulting from the treatment of PTK corneal haze with mitomycin: a case report. Cornea 2004;23:744–747. [PubMed: 15448508]