Concentrations of Mitomycin C in Rabbit Corneal Tissue and Aqueous Humor After Topical Administration

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Purpose: To study the aqueous and corneal pharmacokinetics of mitomycin C (MMC) after single topical administration to the central cornea and to evaluate the effects of different concentrations and different application times on the aqueous concentration of MMC.

Methods: Mechanical epithelium debridement of the central 7.5 mm of the cornea was performed in New Zealand white rabbits, and a sponge soaked in 0.02% MMC solution was placed on the denuded corneal stroma for 2 minutes. Aqueous fluid and central corneal tissues samples were taken at 0.5, 1, 2, and 3 hours thereafter. MMC concentration of the samples was analyzed by high-performance liquid chromatography and evaluated at different exposure times (range: 15–120 seconds) and concentrations of applied MMC (range: 0.005%–0.04%).

Results: Peak corneal concentration was 3.728 ± 2.547 μg/g at 30 minutes after topical administration. Maximum aqueous concentration was 0.380 ± 0.038 μg/mL at 1 hour after topical application. The aqueous concentration of MMC increased in a dose-dependent manner with increasing exposure time and application concentration. Aqueous MMC concentration increased at a higher rate with change of applied concentration than with exposure time.

Conclusion: Good penetration of MMC through central bare cornea may be noxious to endothelial cells. Reducing concentration or decreasing exposure time seems a good modality to reduce potential MMC toxicity.

Key Words: mitomycin C, pharmacokinetics, aqueous concentration, cornea, high-performance liquid chromatography

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Mitomycin C (MMC) is known as a potential modulator of wound healing after photorefractive keratectomy (PRK) in experimental models.[1–3] Some authors recently reported clinically successful results of applying topical MMC to eyes with subepithelial fibrosis after PRK or radial keratotomy and in preventing corneal haze after PRK in high myopia.[4,5] Also, prophylactic treatment with MMC has been gaining popularity as a method to reduce clinically significant haze after surface laser ablation.

The mechanism of action of MMC in cornea is controversial. Inhibition of keratocyte proliferation after PRK was early thought to be the main mechanism of MMC, because MMC was shown to have antiproliferative effects on cultured human keratocytes.[6] However, because MMC was recently reported to cause apoptosis of cultured human keratocytes,[7,8] apoptosis of keratocytes has been considered the main mechanism of the MMC effect.[9]

Although there is no clinical report of a decrease in endothelial cell density in normal human cornea after topical MMC treatment combined with surface laser ablation, a case of permanent corneal edema resulting from endothelial cell loss after treatment of topical MMC was recently reported in a patient with basement membrane dystrophy.[10] In a rabbit model, a decrease in endothelial cell density and early corneal edema after topical application of MMC has been reported,[11,12] and early apoptotic endothelial cells were observed.[11]

We studied the aqueous and corneal pharmacokinetics of MMC after topical administration and evaluated the effects of different application times and applied concentrations on the aqueous concentration of MMC to estimate the amount of MMC reaching the endothelial cell layer after topical MMC application.

MATERIALS AND METHODS

Materials

New Zealand white rabbits were used in this study. Use of these rabbits conformed to the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. MMC (Kyowa, Tokyo, Japan) powder was dissolved at various concentrations in saline, and all MMC solutions were freshly prepared immediately before the studies.

Experiment 1: Aqueous and Corneal Pharmacokinetics of MMC

After rabbits were anesthetized by intramuscular injection of ketamine hydrochloride (40 mg/kg body weight), corneal epithelium of the central area (7.5 mm in diameter)

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was manually abraded using a golf-club knife. A sponge 6 mm in diameter soaked in 0.02% MMC solution was placed on the denuded stromal surface for 2 minutes. After removal of the sponge, eyes were irrigated with 30 mL of saline. Approximately 0.1 mL of aqueous fluid was aspirated from the anterior chamber by a 1-mL syringe, and the central cornea was immediately taken by 7-mm vacuum trephine (Katena Products, Denville, NJ) at each of the following time intervals: 0.5, 1, 2, and 3 hours after drug administration. Four eyes were assigned to each time interval. The wet weight of corneal buttons was measured by an electronic weighing machine and samples were stored at –80°C until analysis.

Experiment 2: Effects of Different Application Times and MMC Concentrations

To evaluate the effects of different application times on aqueous MMC concentrations, a sponge soaked in 0.02% MMC solution was placed on the central bare stromal surface for 15, 30, 60, and 120 seconds. Four eyes were assigned to each application time. The rest of the procedure was the same as in Experiment 1. Aqueous fluid was aspirated at the time of maximum aqueous concentration as observed in Experiment 1. To evaluate the impact of different applied concentrations on aqueous MMC concentrations, sponges were soaked in MMC solutions with concentrations of 0.005%, 0.01%, 0.02%, and 0.04%. Aqueous fluid was also aspirated at the time of peak aqueous concentration. Four eyes were assigned to each concentration.

High-Performance Liquid Chromatography

Each rabbit corneal button was homogenized by a handheld grinder with 4 mL of acetonitrile. The mixture was ultrasonicated for 30 minutes, and the extract was recovered by centrifugation at 3000*g* for 10 minutes. Aqueous solvents were completely evaporated under nitrogen gas at 40°C, and MMC was resolved by 150 μL of 0.02 mol/L phosphate buffer. One hundred microliters of sample was injected into high-performance liquid chromatography (HPLC). For aqueous humor, 3 mL of ethyl acetate was added, and MMC was extracted from 2 aqueous phases by vortexing and centrifugation. The upper phase was recovered and completely dried out under nitrogen gas at 40°C. MMC was resolved by 150 μL of 0.02 mol/L phosphate buffer, and 100 μL of sample was injected into HPLC. A 2-gradient mobile system was used for analysis of MMC: phase A, 10% acetonitrile in 20 mmol/L phosphate buffer; and phase B, 60% acetonitrile in 10 mmol/L phosphate buffer (both pH 7). The gradient time program was 0 to 1 minutes for 100% mobile phase A; 1 to 6.5 minutes for 40% mobile phase A and 60% mobile phase B; and 6.5 to 9 minutes for 100% mobile phase B. The flow rate of the mobile phase was 0.7 mL/min at 30°C, and the injection volume at each time was 100 μL. The liquid chromatographic system consisted of a Hewlett-Packard 1100 model equipped with autosampler and quaternary pumping unit and Hewlett-Packard model DAD G1315A programmable UV detector (Agilent Technologies, Palo Alto, CA). Separation was carried out by 150 × 4.6 mm i.d. 5-μm Zorbax Eclipse XDB-C18 column (Agilent Technologies, Santa Clara, CA). The UV wavelength detector was set at 360 nm. The minimal level of detection was 0.001 μg/mL.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software version 10.1 (Chicago, IL) was used for statistical analysis. A nonparametric Kruskal-Wallis test was used to compare mean aqueous concentrations of MMC at each exposure time and at each applied concentration because there were not enough samples for a parametric test. $P < 0.05$ was considered statistically significant.

RESULTS

Experiment 1

Mean concentrations of MMC at different time intervals after topical administration are shown in Table 1 and Figure 1. The peak corneal concentration of MMC was 3.728 ± 2.547 μg/g at 30 minutes after topical administration. Corneal concentration dropped to 0.756 ± 0.437 μg/g 1 hour after drug administration. However, the aqueous concentration of MMC peaked at 1 hour rather than 30 minutes after topical application. The mean aqueous concentration of MMC was 0.199 ± 0.067 μg/mL at 30 minutes and 0.380 ± 0.038 μg/mL at 1 hour. Although concentrations of MMC were minimal, MMC remained in aqueous humor and corneal tissue less than 3 hours after topical administration.

Experiment 2

According to the result of Experiment 1, aqueous humor was aspirated at 1 hour after topical application. Mean aqueous concentrations of MMC for different application times are shown in Table 2. The aqueous concentration of MMC tended to increase in a dose-dependent manner with increasing exposure time (Fig. 2A). However, concentration changes for different exposure times were not statistically significant ($P = 0.22$). Table 3 shows mean aqueous concentrations of MMC for different applied concentrations. As the applied concentration increased, the aqueous concentration increased in a dose-dependent manner (Fig. 2B), and this change for different concentrations was statistically significant ($P < 0.01$). Aqueous MMC concentration increased at a higher rate along with change of applied concentration than with change of exposure time.

DISCUSSION

Since some cases of iatrogenic keratectasia after laser in situ keratomileusis (LASIK) were reported,[13–16] increased

TABLE 1. MMC Concentrations in Corneal Tissue and Aqueous Humor After Topical Administration (Mean ± SD)

Interval (h)	Corneal Concentration (μg/g)	Aqueous Concentration (μg/mL)
0.5	3.728 ± 2.547	0.199 ± 0.067
1	0.756 ± 0.437	0.380 ± 0.038
2	0.049 ± 0.012	0.024 ± 0.010
3	0.038 ± 0.001	0.017 ± 0.010

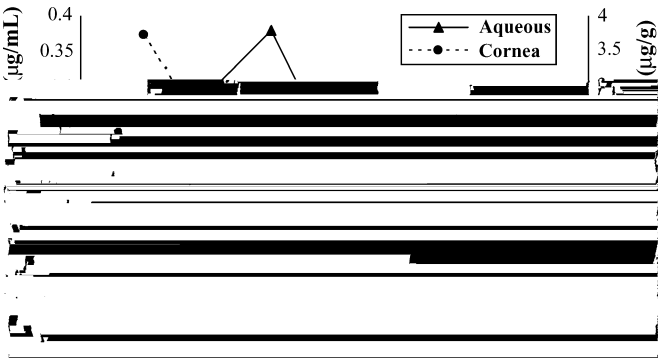

FIGURE 1. MMC concentrations in aqueous humor and corneal tissue at each time interval after topical administration.

attention has been paid to residual corneal thickness, and renewed interest has arisen in surface laser ablation such as PRK, laser subepithelial keratomileusis (LASEK), and Epi-LASIK to increase remaining corneal thickness. However, haze formation is still a major complication of surface laser ablation, especially for high myopia. Much research has been focused on finding the most effective prophylactic treatment.

Topical MMC was first used to treat human corneas with subepithelial fibrosis after PRK or radial keratotomy.[4] The authors of that report suggested that topical application of MMC might be a successful method of preventing the recurrence of subepithelial fibrosis. Thereafter, Carones et al[5] clinically evaluated the prophylactic effects of MMC on haze formation after PRK and found that the prophylactic use of MMC produced lower haze rates, better uncorrected and the best corrected visual acuity, and a more accurate refractive outcome than those achieved without MMC treatment. A confocal microscopic study recently showed that activated keratocytes and the extracellular matrix were more evident in untreated eyes than in MMC-treated eyes.[17]

Although MMC was originally used as a systemic chemotherapeutic agent, topical MMC has been used with increasing frequency for ophthalmic indications. Topical MMC has become popular in glaucoma filtering surgery,[18] pterygium surgery,[19,20] and the treatment of conjunctival and corneal intraepithelial neoplasia.[21,22] However, severe ocular complications including corneal edema, corneal perforation, and scleral melting have been reported after MMC use in pterygium surgery.[21,23]

TABLE 2. Aqueous Concentrations of MMC for Different Exposure Time (Mean ± SD)

Exposure Time (s)	Aqueous Concentration (μg/mL)
15	0.082 ± 0.075
30	0.108 ± 0.063
60	0.153 ± 0.142
120	0.199 ± 0.051

Although there is no clinical report of corneal complications after prophylactic MMC use combined with surface laser ablation, a few experimental studies reported early corneal edema in rabbit eyes after topical MMC application.[11,12] Early apoptotic changes in the endothelial cells of rabbit cornea were also shown.[11] MMC's effects on human cornea could be different from those on rabbit cornea because the rabbit cornea does not have a Bowman membrane and is thinner than the human cornea. Furthermore, rabbit corneal endothelium is capable of mitosis.[11] Except for endothelial mitotic capacity, however, the rabbit cornea is quite similar to a markedly ablated human cornea in terms of the absence of Bowman membrane and similar corneal thickness. Therefore, we must be mindful of possible corneal changes that may occur after topical MMC administration in high-myopic patients.

To the best of our knowledge, this is the first study that investigated aqueous and corneal pharmacokinetics of MMC after topical application to central bare cornea. This topical application is the same method that we have used clinically for preventing corneal haze after surface laser ablation. The maximum corneal concentration of MMC was obtained at 30 minutes and aqueous concentration peaked at 1 hour after topical administration. Although the concentration was minimal, MMC remained in aqueous humor and corneal tissue for less than 3 hours after topical administration. There have been a few studies of MMC concentration in ocular tissues after topical application[24–27]; however, in these reports, the MMC-administering methods were different from that of this study. Sarraf et al[25] applied cellulose sponges soaked in 0.04% MMC solution on conjunctival bleb for 5 minutes to study the aqueous concentration of MMC in rabbit eyes that had undergone filtering surgery. Peak aqueous concentration was achieved at 1 hour after administration, which is identical to

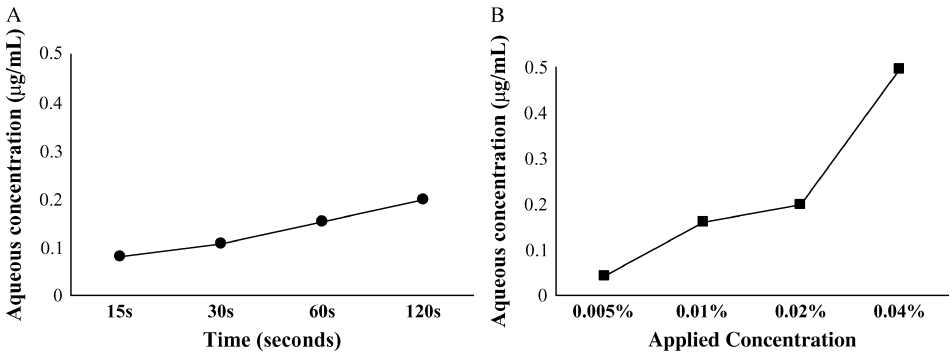

FIGURE 2. Changes of aqueous MMC concentration with increasing exposure time (A) and increasing applied concentration (B).

TABLE 3. Aqueous Concentrations of MMC for Different Applied Concentrations (Mean ± SD)

Applied Concentration (%)	Aqueous Concentration (μg/mL)
0.005	0.043 ± 0.012
0.01	0.161 ± 0.048
0.02	0.199 ± 0.051
0.04	0.496 ± 0.289

our findings. However, despite a higher MMC concentration and longer application time, mean aqueous concentration was lower in that study than in ours. The penetration of MMC through the central bare cornea seems to be more effective than that through the conjunctiva and sclera, and this application method may have a higher risk of endothelial cell damage.

The currently recommended MMC treatment of preventing corneal haze is performed by placing a 0.02% MMC-soaked sponge over ablated cornea for 2 minutes.[5] However, to reduce potential MMC toxicity, there is a trend to use lower concentrations of MMC for shorter times. Camellin[28] used 0.01% MMC on LASEK-treated corneas and thereby successfully decreased subepithelial haze. Using 0.002% MMC, Netto et al[29] showed the effective prevention of corneal haze formation in rabbit eyes with exposure times ranging from 12 seconds to 2 minutes. However, 0.002% MMC was not as effective in treating existing corneal haze.[29] As this study shows, aqueous MMC concentration decreased in a dose-dependent manner with decreasing concentration and application time. Reducing concentration or decreasing exposure time seems a good modality to reduce possible endothelial toxicity. According to our study, alteration of concentration is more effective in reducing aqueous concentration than alteration of exposure time. Further studies are needed to find ideal combinations of concentration and application time that are effective in preventing corneal haze and minimally toxic to corneal tissue.

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