Mitomycin C aqueous humor concentration after photorefractive keratectomy: an experimental study

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PURPOSE. To evaluate mitomycin C (MMC) aqueous humor concentration after photorefractive keratectomy (PRK).

METHODS. In this experimental study, twenty-four eyes of 12 male pigmented rabbits were divided into 4 groups and studied at the Institute of Vision and Optics, Department of Medicine, University of Crete, Greece. Eyes in groups 1 and 2 underwent PRK to correct –5 diopters (D) in a 6-mm optical zone, while sponges soaked with 0.02% MMC were applied on the exposed corneal stroma for 60 and 120 seconds, respectively. Similarly, eyes in groups 3 and 4 underwent PRK to correct –10 D in a 6-mm optical zone, while sponges soaked with 0.02% MMC were applied on the exposed corneal stroma for 60 and 120 seconds, respectively. Aqueous humor was extracted from all rabbit eyes 10 minutes after MMC application and high-performance liquid chromatography was performed immediately to detect and quantify MMC levels.

RESULTS. The mean aqueous humor concentration of MMC was 0.23±0.03 μg/mL, 0.39±0.05 μg/mL, 0.28±0.04 μg/mL, and 0.52±0.16 μg/mL in groups 1, 2, 3, and 4, respectively. The effect of application time and correction on aqueous humor MMC concentration was significant (p<0.0001 and p=0.019), while the exposure time had a greater impact on aqueous humor MMC concentration when compared with the attempted correction.

CONCLUSIONS. Both exposure time of MMC on the corneal stroma and the attempted correction was correlated with MMC aqueous humor concentrations. (Eur J Ophthalmol 2009; 19: 738-42)

KEY WORDS. Photorefractive keratectomy, Mitomycin, Experimental study, Aqueous humor

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INTRODUCTION

Mitomycin belongs to a group of medicines called cyto-toxic antibiotics. These are synthetic medicines that have been derived from compounds of certain bacteria and fungi. Mitomycin acts as an alkylating agent that inhibits DNA and protein synthesis by inserting itself into the strands of genetic material. Consequently, proliferation of rapidly growing cells such as fibroblasts is inhibited, causing cell apoptosis. Attributable to its inhibiting properties, MMC has been used in ophthalmology for over 20 years as an adjunctive treatment of a variety of ophthalmic conditions. Improvements in the outcomes of trabeculectomy (1), pterygium surgery (2), and corneal intraepithelial neoplasia (3) after the application of MMC have been reported extensively.

Lately, MMC has been used intraoperatively after PRK to reduce the incidence and severity of haze formation by inhibiting fibroblastic proliferation and inducing keratocyte apoptosis (4-8). Despite the inhibition of haze formation by
the cytostatic action of MMC, controversy regarding its adverse effects on different ocular tissues and its long-term safety have been raised (9-15).

In this experimental study, we investigated the correlation of aqueous humor MMC concentration with application time and the residual corneal thickness after photorefractive keratectomy.

MATERIALS AND METHODS

Animals

Twenty-four eyes of 12 male pigmented rabbits weighting 2.5–3.5 kg were used in this study. All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Research, while the design of the study was approved by the regional Animal Care Committee. The animals were divided into 4 groups (6 eyes per group) according to the refractive treatment (−5 Diopters [D] and −10 D) and the time interval of MMC application on the exposed corneal stroma (60 and 120 seconds). Groups 1 and 2 underwent PRK to correct −5 D in a 6-mm optical zone, while sponges soaked with MMC (0.02%) were applied on the exposed corneal stroma for 60 and 120 seconds, respectively. Similarly, groups 3 and 4 underwent PRK to correct −10 D in a 6-mm optical zone with MMC exposure time of 60 and 120 seconds, respectively.

Prior to any procedure, all animals were anesthetized by an intramuscular injection of a mixture of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (50 mg/kg) and draped; an eyelid speculum was placed to separate the lids, and 2 drops of topical anesthesia were instilled in the conjunctival fornix. Aqueous humor was extracted from all rabbit eyes 10 minutes after MMC application and high-performance liquid chromatography was performed to detect and quantify MMC levels.

Surgical technique

All PRK procedures followed the same surgical technique. Two minutes after topical corneal anesthesia, mechanical epithelium debridement of the central 7.5 mm of the cornea (previously marked with a 7.5 mm trephine) was performed using a rotating soft brush followed by myopic photoablation with the Wavelight Allegretto 400 (fluence of 180 mJ/cm² per pulse at 400 Hz). The rabbits' mean central corneal thicknesses were measured before epithelial removal and after the photoablation with an ultrasonic pachymeter (Corneo-Gage Plus, Sonogage Inc.). Immediately after photoablation, a cellulose surgical sponge (5 mm dry size) soaked in 1 mL of MMC 0.2 mg/mL (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) was applied over the exposed corneal stroma for 60 seconds (groups 1 and 3) or 120 seconds (groups 2 and 4). To limit the surface of MMC exposure, the application was carried inside a 5.5-mm trephine. The sponge was then removed, followed by an immediate washing with 20 mL of balanced salt solution.

Aqueous humor extraction

Aqueous humor was extracted 10 minutes after MMC treatment from all eyes. A 30-gauge needle entered in the anterior chamber from the limbus (outside the MMC treatment zone) to avoid a potential error in surface collection of MMC during the procedure. Aqueous humor was placed into an Eppendorf tube and the samples were immediately tested by high performance liquid chromatography (HPLC) to measure MMC concentration.

Preparation of standard curves

Stock solutions containing MMC were prepared in water at 0.02% μg/mL and stored at 4 °C. Seven diluted solutions were prepared at concentrations of 0.00, 0.10, 0.25, 0.50, 1.00, 2.50, and 5.00 μg/mL. The curve was linear with $r^2=0.9996$. Detection limit (LOD) was calculated in the standard solutions at 0.10 μg/mL.

MMC detection procedure

The samples collected from each rabbit (50 to100 μL of aqueous humor) were placed in Eppendorf tubes (2 mL), vortex from 5 sec, and centrifuged at 14,000 rpm for 3 min. The injection volume was 40 μL. A reversed-phase HPLC method was carried out on a Spectra Physics (Spectra Physics 8800, San Jose, CA) using a Nucleosil 100, C18, 5 μm, 250 mm × 4.6 mm ID column (Rigas Lab, Thessaloniki, Greece). The mobile phase consisted of methanol:water (35:65) with 0.01% TFA (16). The elution conditions were isocratic and the mobile phase flow-rate was set at 0.7 mL/
MMC aqueous humor concentration after PRK in rabbit eyes

mL. UV absorbance detection at 365 nm was carried out with a UV/VIS detector (Spectra Physics 8450, San Jose, CA) and the range of the detector was set at 0.01 AUFS. Under these conditions, the retention time of mitomycin C was 10.8 min.

Statistical analysis

To evaluate the effects of attempted corrections and application times on aqueous MMC concentrations, a general linear regression model was used with MMC concentration as the dependent variable and correction and time as independent variables. In addition, interaction effects between the correction and time application were also included in the model. All statistical analysis was performed using JMP 5.01 statistical software.

RESULTS

Mean residual corneal bed thickness (RCBT) after epithelial layer removal (estimated 35 μm) and stromal ablation was 233.5±14.13 μm, 241.83±13.02 μm, 170±45.42 μm, and 162.33±29.96 μm for groups 1, 2, 3, and 4, respectively. The mean aqueous humor concentration of MMC was 0.23±0.03 μg/mL, 0.39±0.05 μg/mL, 0.28±0.04 μg/mL, and 0.52±0.16 μg/mL for groups 1, 2, 3, and 4, respectively (Tab. I).

Both RCBT (attempted correction) and MMC application time had a significant effect on the aqueous MMC concentration (p=0.019 and p<0.0001, respectively), while their interaction had no statistically significant effect on the model (p=0.2843). Moreover, the regression parameter of application time (~0.1 μg/mL) was higher in absolute terms than the regression parameter of attempted correction (~0.045 μg/mL), indicating an aqueous MMC concentration difference between the two time groups (0.20 μg/mL, 95% CI 0.13–0.27 μg/mL) higher than the difference between the two correction groups (0.09 μg/mL, 95% CI 0.017–0.17 μg/mL).

DISCUSSION

Nine years after experimental studies on rabbit corneas (17), the first clinical study of PRK with adjuvant MMC in 2001 demonstrated satisfactory refractive outcomes by modulating the corneal healing response and controlling haze formation (18). Despite the early findings of satisfactory and stable refractive results and the prophylactic role of MMC, severe ocular complications have been reported after use in pterygium surgery (10), providing a considerable concern about its level of penetration and adverse effects in refractive surgery. Even though there are clinical and experimental reports demonstrating no adverse effects of MMC when used in PRK (11, 19), it is useful to know the factors that could contribute to an increased penetration of MMC in the aqueous humor, in the context of refractive surgery. Recently, Torres et al (on hen eyes) (20) and Song et al (on rabbit eyes) (21) provided evidence that MMC enters the anterior chamber after topical application, while the penetration level of the agent was correlated with its concentration and application time interval onto the cornea.

In the present study, we report a significant dependence of the aqueous humor MMC concentration on time application. Additionally, we investigated the effect of attempted correction on the aqueous humor MMC concentration and found a statistical influence on the agent’s concentrations that has not been reported. In contrast to the previous studies, the levels of aqueous humor MMC concentration were higher, probably related to the changes of the corneal pachymetry after the ablation and/or the different extraction times and animals used. These findings are critical as MMC comes in immediate contact with different ocular tissues and the short-term or long-term effects are not well established.

In our study, we extracted aqueous humor from rabbit anterior chamber 10 minutes after topical application. Because the aim of this report is to study the correlation between the exposure time of MMC and the attempted correction with aqueous humor MMC concentrations, the extraction time point is a constant not significant to our purpose. There-

<table>
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<th>Group number</th>
<th>MMC concentration (μg/mL)</th>
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<tr>
<td>1</td>
<td>0.23±0.03</td>
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<tr>
<td>2</td>
<td>0.39±0.05</td>
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<tr>
<td>3</td>
<td>0.28±0.04</td>
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<tr>
<td>4</td>
<td>0.52±0.16</td>
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fore, we did not select as extraction time the 60-minute interval which is the peak of aqueous MMC concentration (rabbit model) after topical application as proved in other studies (21).

A review of the latest reports reveals a tendency with respect to the concentration of MMC used in surface ablations by the photorefractive community (7, 8, 11, 22). Unlike this common concentration of 0.02% MMC, the exposure time of MMC on the ablated stromal surface varies. Even though an application time up to 120 seconds has been reported (11, 13, 22), most surgeons do not exceed an application time of 45 seconds. In our study, we choose MMC application of 60 and 120 seconds (extreme and distant time intervals) in order to demonstrate relevance between MMC penetration and intraoperative application; this can be considered as a limitation since such application time intervals are not common in everyday clinical practice. The lack of standardization in concentration of MMC along with the significant effect of application time and attempted correction on aqueous humor MMC concentration exemplify the need for further studies examining all three variables along with haze formation to establish a regression model describing the complete effect.

An important limitation of this study is that rabbit corneas are approximately half in pachymetry in comparison with humans, while corneal anatomy differs between the two species (rabbit cornea does not have a Bowman membrane). These factors may influence the penetration of MMC into the anterior chamber and an extrapolation of this study finding to humans is difficult. Nevertheless, when correcting ametropias during laser refractive procedures, corneal tissue is removed in the process, changing corneal properties in terms of drug penetration (23).

In conclusion, both the application time of MMC and RCBT-attempted corrections affect MMC concentrations in the aqueous humor after PRK. These parameters along with MMC applied solution should be taken into consideration in order to minimize MMC penetration in the anterior chamber.

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REFERENCES


