Effect of intraoperative mitomycin-C application on epithelial regeneration after transepithelial photorefractive keratectomy

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Running head: Effect of MMC on the epithelial regeneration after TransPRK

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ABSTRACT

Purpose: To investigate the effect of Mitomycin C (MMC) intraoperative application on postoperative corneal epithelial remodeling, haze incidence and refractive correction in Transepithelial Photorefractive Keratectomy (TransPRK).

Setting:

Eye Hospital of Wenzhou Medical University, Wenzhou, China.

Design:

Prospective randomized controlled study.

Methods: A total of 100 eyes completed all follow-up were included, including 40 eyes treated with MMC in MMC group, 60 eyes without treated with MMC in control group. Epithelial thickness (ET) map measurement was conducted preoperatively and first week, first, third and sixth months postoperative, generating ET in central, paracentral, midperipheral zones. The difference between postoperative and preoperative ET (ΔET) was computed for each zone. During follow-up, haze incidence and visual acuity were assessed and compared between groups.

Results: For ΔET, between-group difference was found in the midperipheral ($P = 0.011$) zone at first week postoperative and in the central ($P = 0.036$) and paracentral zones ($P = 0.039$) at first month postoperative. Haze incidence was lower in MMC group at first week and first month postoperative ($P = 0.035$ and 0.018, respectively). Safety Index (postoperative uncorrected distance visual acuity/preoperative corrected distance visual acuity (CDVA)) and Efficacy Index (postoperative CDVA / preoperative CDVA) were higher in MMC group ($P = 0.012$ and $P = 0.036$, respectively) at first month

postoperative. No difference was found after third months postoperative.

Conclusions: With a temporary impact on corneal epithelial regeneration and refractive correction, intraoperative MMC application in TransPRK decreased the haze incidence but had no effect on epithelial remodeling.

INTRODUCTION

The adjuvant application of Mitomycin C (MMC) in corneal laser refractive surgery has been described more than 20 years. [1] Numerous studies have reported outcomes of intraoperative use of MMC in photorefractive keratectomy (PRK) [2-5] and laser epithelial keratomileusis (LASEK). [6-10] TransPRK using SmartPulse Technology (SPT) combines defined-depth phototherapeutic keratectomy with radial compensation and PRK in one single step, achieving an evenly distributed laser ablation, contributing to a faster recovery of visual acuity and regeneration of the epithelium than conventional PRK [11, 12]. MMC is a potent mitotic inhibitor which preferentially affects rapidly proliferating cells [13] like keratocytes and consequently has an impact on epithelial regeneration. To the best of our knowledge, no study has reported the influence of MMC on epithelial remodeling after TransPRK by objective evaluation from optical coherence tomography (OCT).

The purpose of this study was to investigate the effect of MMC on the postoperative epithelial regeneration TransPRK, the incidence of haze, and the refractive correction effectiveness.

PATIENTS AND METHODS

Patients

In this prospective study, patients were enrolled from Eye Hospital of Wenzhou Medical University. The study protocol conformed to the tenets of Declaration of Helsinki and was approved by the Office of Research Ethics, Eye Hospital of Wenzhou Medical University. All enrolled participants have submitted written informed consent.

The inclusion criteria comprised age between 18 and 40 years, documented refractive stability for more than 2 years, myopia $\leqslant$ -12.00 diopters (D) and astigmatism $\leqslant$ -3.00 D, and corrected distance visual acuity (CDVA) $\geq$ 18/20 for both eyes. The patients had to stop wearing soft contact lenses for at least two weeks and hard contact lenses for at least four weeks prior to the preoperative examination. The exclusion criteria included pregnancy or lactation, severe dry eye (the result of Schirmer's test$<$4mm), previous ocular or corneal surgery, keratoconus or ectasia and other active ocular diseases other than myopia. The patients were randomly assigned into either the MMC group or the control group by random number table method.

Examination

All patients underwent comprehensive ophthalmologic evaluations preoperatively and postoperatively, including anterior segment spectral-domain optical coherence tomography (OCT) (RTVue-XR; Optovue, Inc., Fremont, CA), eye tonometer (TX-20, Canon, Japan), corneal haze evaluation with slit lamp biomicroscopy by 2 experienced ophthalmologists according to the system described by Fantes et al. and Fadlallah et al. [14, 15], uncorrected distance visual acuity (UDVA) and subjective refraction for

CDVA, Scheimpflug-based corneal topography (Pentacam® HR, Oculus Optikgeräte, Wetzlar, Germany), Placido-based corneal topography and wavefront aberrometry (Optikon 2000 SPA, Rome, Italy), Corneal Visualization Scheimpflug Technology tonometer (Corvis ST tonometry: CST; Oculus, Wetzlar, Germany).

OCT Epithelial Thickness Measurement

Three consecutive measurements were preoperatively and postoperatively conducted in "PachymetryWide" scan mode by the same operator using RTVue-XR. The data was utilized to analyze if the outcomes of measurement had enough image signals without mosaic simulation area on images. Regarding to the following four zones, the data of epithelial thickness (ET) and stroma thickness (ST) were recorded: (1) The central region of two mm diameter, (2) Eight paracentral regions of two to five mm diameter, (3) Eight midperipheral regions of five to seven mm diameter [16].

Surgical Technique

The custom ablation manager (version 5.2.23, SCHWIND eye-tech-solutions GmbH & Co., Kleinostheim, Germany) and SCHWIND Combi Wavefront Analyzer (workstation 9.0-6) were responsible for the design of ablation profile. For the epithelial ablation, the ablation targeted the average preoperative ET centrally, and corresponding added 10 μm peripherally applied. The polyvinyl alcohol sponge soaked with 0.02% MMC was prepared.

During the ablation procedures, proparacaine hydrochloride (0.5%Alcaine®, Alcon) was applied and redundant liquid was wiped with semi-dry sponge pad. All ablations were performed using the Amaris 750S excimer laser system (Schwind eye-

tech-solutions, GmbH, Kleinostheim, Germany) according to the design. Dynamic and static cyclorotation controls has also been implemented in all eyes.

The control group had both eyes irrigated immediately with 30 mL 4℃ Ringer's Solution. For the MMC group, the prepared sponge was squeezed with forceps and coated evenly on the ablated cornea. The surgical assistant set a timer for the applying time of MMC on the residual stromal bed followed by irrigation with 30 mL 4℃ Ringer's Solution. As shown in Table 1, the time coincided with the actual depth of ablation obtained from the software.

One drop of Tobramycin Dexamethasone (Tobradex®, Alcon Laboratories, Inc., Fort Worth, TX) was instilled and then a soft bandage contact lens (ACUVUE OASYS® Brand Contact Lenses with HYDRACLEAR® PLUS, Johnson & Johnson) was placed on the cornea.

Postoperative Medication Regimen

The bandage contact lens was removed when epithelial healing was accomplished. The medications consisted of artificial tears and glucocorticoid drops that were both used four times daily during the first week postoperative. The glucocorticoid drops, fluorometholone 0.1% eye drop (FML; Allergan, Irvine, CA, USA), were prescribed for four months: four times a day in the first month postoperative, and then were tapered one drop a month. Aqueous replacement drops were used as needed.

Statistical Analysis

The ΔET was defined as the subtraction of postoperative regional ET from preoperative corresponding regional ET. The mean ΔET of each of eight regions (nasal-

inferior, nasal, nasal-superior, superior, temporal-superior, temporal, temporal-inferior, inferior regions) was compared between the two groups. The Safety Index (SI) was defined as the postoperative UDVA / preoperative CDVA and Efficacy Index (EI) as the postoperative CDVA / preoperative CDVA.

Shapiro-Wilk test was applied to verified the normality of data distribution. Depending on the normality, the data was statistically described as mean ± standard deviation or median (lower quartile, upper quartile), and Student's t test or Mann-Whitney U test was used to analyze the difference in continuous variables between the MMC group and the control group was analyzed using. The incidence of haze between groups was compared using Mann-Whitney U test. For verifying the epithelial remodeling, one-sample t test was used to compare the ΔET at sixth months postoperative and a value of 0. All statistical analyses were performed using the SciPy (version 1.2.0) based on Python (version 3.7.0).

RESULTS

100 patients who underwent surgeries and completed all the scheduled follow-up visits were recruited. For each patient, one eye was randomly selected resulting in 24 right eyes and 16 left eyes in the MMC group and 33 right eyes and 27 left eyes in the control group.

The preoperative data and surgical parameters are shown in Table 2. The mean depth of intended ablation, including the epithelial component, was 144.1 ± 20.4 μm (range, 101–189 μm) in the MMC group and 147.27 ± 23.14 μm (range, 102–196 μm)

in the control group. (P = 0.484). Median optical zone was 6.4 mm (5.8, 6.7; range, 5.40–7.10 mm) in the MMC group and 6.5 mm (6.2, 6.7; range 5.7–7.1 mm) in the control group (P = 0.151).

Visual and Haze Outcomes

As shown in Table 3 (A), at the first week postoperative, the CDVA appeared worse for MMC group with a lower SI, although there was no statistical difference. At first month postoperative, the UDVA (LogMAR) and CDVA (LogMAR) were significantly worse for the MMC group, with significantly higher SI and EI. At the third and sixth month postoperative, no statistical difference was found for all indices. All eyes had good and stable visual and refractive results and no eye lost two or more lines of visual acuity at sixth months postoperative (Figure 1 and Figure 2).

As shown in Table 3 (B), no stromal haze worse than grade 1 has been found during all the follow-up visit. Compared with the control group, the incidence rate of haze in MMC group was statistically lower for first week and first month postoperative.

Preoperative and Postoperative Profile of Epithelial Thickness

Figure 2 displayed the ΔET for different regions.

As shown in Table 4, the ΔET at midperipheral zone of 5 to 7 mm (P = 0.011) between groups in first week postoperative was significantly different. Contrarily to this, the statistically significant difference in the central 2mm region (P = 0.036) and paracentral zone within 2 to 5 mm (P = 0.039) was observed at first month postoperative. By third month postoperative, there was no difference in ΔET between the two groups.

For both the MMC and control groups, ΔET values at sixth months postoperative

were statistically different from 0 for central (P = 0.040 for MMC group and 0.001 for control group) and paracentral zones (P = 0.001 for MMC group and less than 0.001 for control group) tested by one-sample t test, suggesting the epithelial remodeling in central and paracentral zones. Nevertheless, no statistical significance was found for the peripheral zone (P = 0.317 for MMC group and 0.703 for control group).

DISCUSSION

In the present study, a lower incidence rate of haze for TransPRK in the MMC group until the first month postoperative was observed, indicating that MMC was also successful in preventing corneal haze following this improved surface ablation modality from PRK, and suggesting that more perfect ablation algorithm design is needed to reduce haze incidence. However, Adib-Moghaddam et al. [10] did not find this difference between the MMC group and the controls among patients with mild to moderate myopia. This attributed to the different average ablation volume reported by Moller-Pedersen et al. that haze production is that in proportion to the depth of stroma ablation [17], since the SE was around -6.00 D with an ablation depth of 150 μm in our study [3]. Torricelli et al. [18] found that high volume ablated cornea has slower reformation and less integrity of epithelial basement membrane (EBM) which stimulates the development of haze. Consequently, it's not surprising that the lower ablation volume itself decreased haze production and did not reflect the value of MMC, leading to negative findings in the study by Adib-Moghaddam.

In the MMC group, although a lower incidence rate of haze was found at the first month postoperative, the UDVA (LogMAR), CDVA (LogMAR), SI and EI were worse.

On the third and sixth months postoperative, both groups had comparable and better visual acuity than their early-stage. The lower incidence of haze with poor visual indexes in the MMC group at first month postoperative indicated that this most likely contributed to the different recovery process and the temporary toxic effects of MMC on fibroblasts and myofibroblast.

At first week postoperative, the ΔET in the MMC group at mid-peripheral zone from 5 to 7 mm was less than the controls. After surgery, the corneal epithelial stem cells at the limbus give rise to transient amplifying cells, which migrate toward the central area and generate basal cells to form EBM, then the basal cells proliferate and differentiate into wing cells and superficial cells. The intraoperative therapy of MMC impacted this regeneration by combining DNA with a covalent linkage and thus inhibited DNA synthesis which triggered apoptosis and inhibited the proliferation of all cells. No difference observed in the central and paracentral ΔET at first week postoperative was probably because, given that the epithelial recovery started from the periphery, the speed of epithelial regeneration was still slow for the control group to generate the difference within 5-mm area. At first month postoperative, owing to the effect of MMC, the regeneration of central and paracentral epithelial cells in the MMC group was slower than the control group leading the difference of ΔET at the central and paracentral zones. Kremer et al. [19] demonstrated that compared to no MMC application, 20 seconds 0.02% MMC intraoperative application in PRK had more delayed epithelial healing up to 14 days. Rajan et al. [20] proved in experimental model that longer exposure time of MMC is marked by more decreased epithelial migration

rate and epithelial healing. We performed a much longer MMC application than Kremer's study; in view of Rajan's finding, this effect has even postponed to the first month postoperative in the current study. There was no difference in ΔET and visual acuity between the two groups during the third and sixth month postoperative, indicating that the effect of MMC was temporary and did not decrease postoperative epithelial hyperplasia.

The temporary difference in epithelial regeneration explained the above-mentioned phenomenon of poor vision indexes in the MMC group despite lower haze rate: epithelial proliferation was delayed and irregular in the central 5 mm optical zone after application of MMC, resulting in a non-smooth optical section and lower vision indexes. Medeiros et al. [21] identified that, 30 seconds 0.02% MMC application in combination with PRK in the rabbit model exerted an additive toxic effect on the corneal nerves which was significant until first month postoperative. This finding also explain why the poor vision indexes appeared in the MMC group at first month postoperative in the current study.

The concentration of MMC used in PRK ranged from 0.002% to 0.02%, [4, 22, 23] but only a concentration of 0.02% was reported in TransPRK [10]. Depending on the concentration, The MMC-treatment time was set from 10 seconds to 2 minutes [10, 24]. Contrary to some surgeons using constant time and concentration of MMC for variant ablation depth, the duration of interoperative application of MMC in the current study differed according to ablation depth with constant concentration (0.02 %). While the prolonged time of MMC was applied, there was no difference in the epithelium

remodeling of two study groups after the first postoperative month, and no decompensation such as corneal edema was observed during all follow-up periods to prove the safety of long-term application.

At sixth months postoperative, the epithelial remodeling was observed in both groups in central and paracentral zones. Hou et al. [25] firstly used "Pachymetry + Cpwr" model of RTVue to observe the epithelial remodeling up to 6-mm diameter in TransPRK without MMC. Compared to the control group in our work (5.85 ± 1.91 D), the preoperative spherical equivalent in their study was lower (3.96 ± 1.30 D), which might be responsible for this discrepancy. The remodeling pattern was that all zones were hyperplasia postoperative especially in paracentral zone, which was also observed by Kang et al. [26] and Sedaghat et al. [27]. The latter contributed this remodeling to the pre-compensation of paracentral spherical aberration.

A limitation should be noted that the epithelium within 7-9 mm was not included for analysis in current study, as the comparatively lower repeatability of postoperative ET measurement in peripheral zone may have an impact on pachymetric measurement [16].

In summary, while intraoperative use of MMC in TransPRK decreased the occurrence of postoperative haze, worse visual acuity occurs in the initial span of the MMC-treated eyes. Only a temporary effect on postoperative epithelial remodeling has been observed; suggesting intraoperative use of MMC does not affect postoperative epithelial remodeling.

Value Statement

What Was Known

MMC can reduce the occurrence of haze after various corneal refractive surgery.

TransPRK that incorporates SPT technology has further declined the incidence rate of haze.

WHAT THIS PAPER ADDS

The customized application of 0.02% MMC during TransPRK surgery can still reduce the occurrence of haze.

MMC reduces the initial span of visual acuity after TransPRK by temporarily affecting the postoperative corneal epithelial hyperplasia, but it does not affect the remodeling of the postoperative corneal epithelium.

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Figure Legend

Figure 1. Standard graphs for refractive visual outcomes of eyes treated by TransPRK without or with mitomycin C (MMC). The figure demonstrates results of: (A1) and (A2) postoperative uncorrected distance visual acuity (UDVA) vs preoperative corrected distance visual acuity (CDVA) in control group and MMC group respectively; (B1) and (B2) change of CDVA in terms of numbers of decimal lines in control group and MMC group respectively; (C1) and (C2) spherical equivalent correction attempted vs achieved in control group and MMC group respectively; (D1) and (D2) accuracy of spherical equivalent correction in control group and MMC group respectively; (E1) and (E2) accuracy of refractive astigmatism correction in control group and MMC group respectively; and (F1) and (F2) stability of postoperative spherical equivalent refraction in control group and MMC group respectively. D = diopters

Figure 2. The difference of postoperative and preoperative corneal epithelial thickness (ΔET) at first week postoperative for MMC group (A) and control group (B), at first month postoperative for MMC group (C) and control group (D), at third months postoperative for MMC group (E) and control group (F) and at sixth months postoperative for MMC group (G) and control group (H).