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Effects of mitomycin C at different exposure time on the morphology of corneal endothelial cells in LASEK surgery

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丝裂霉素不同作用时间对 LASEK 术后角膜内皮细胞形态的影响

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摘要

目的:研究准分子激光上皮瓣下角膜磨镶术(LASEK)中丝裂霉素 C(MMC)的不同作用时间对角膜内皮细胞的影响。

方法:收集我院行 LASEK 手术的患者,根据丝裂霉素在角膜基质上的作用时间(t),将研究对象分为 T1、T2 两组,其中 T1 组为 15s≤t≤45s,T2 组为 45s<t≤70s。分别于术前及术后 1wk, 1、3、6mo 测定角膜中央及周边区域内皮细胞密度(Cell density, CD)、平均细胞面积(average cell area, ACA)、面积标准差(area standard deviation, SD)、变异系数(coefficient of variation, CV)和六边形细胞百分比(percentage of hexagonal cells, HEX),分析对比两组间这些指标的变化情况。

结果:本研究共纳入患者 98 例 196 眼,T1 与 T2 组分别纳入 98 眼。周边角膜内皮细胞的 ACA、中央角膜内皮细胞的 CV 和 HEX 于术后 1wk 与术前相比差异有统计学意义(P<0.05),其余各指标在各随访时间两组间差异无统计学意义(P>0.05)。

结论:LASEK 术后短期内出现的中央角膜内皮六边形细胞百分比降低、变异系数升高、周边角膜内皮细胞平均面积增大,均为一过性改变。因此,在合理时间范围内应用 MMC,不会对远期角膜内皮状态造成显著影响。

关键词:准分子激光上皮瓣下角膜磨镶术;丝裂霉素 C;角膜内皮细胞

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Abstract

• AIM: To investigate the effects of mitomycin C (MMC) on corneal endothelial cells at different exposure time during laser-assisted subepithelial keratomileusis (LASEK).

• METHODS: Patients who received LASEK were included and divided into Group T1 (15s≤t≤45s) and T2 (5s<t≤70s) based on the exposure time of MMC with corneal stroma. Cell density (CD) of corneal endothelial cells in central and peripheral cornea, average cell area (ACA), area standard deviation (SD), coefficient of variation (CV) and percentage of hexagonal cells (HEX) before surgery and at 1wk, 1, 3 and 6mo after surgery were compared between and within the groups.

• RESULTS: A total of 196 eyes of 98 patients were included with 98 eyes in Group T1 and 98 eyes in Group T2. With the exception of peripheral ACA, central CV and HEX in Group T1 and T2, which significantly changed at 1wk after the surgery (P<0.05), there was no significant difference in central and peripheral parameters within both groups or between the groups (P>0.05).

• CONCLUSION: Although transient acute changes in central CV and HEX and peripheral ACA were observed, there is no significant effect on the long-term corneal health status when MMC is applied in a reasonable time range in LASEK surgery.

• KEYWORDS: laser-assisted subepithelial keratomileusis; mitomycin C; corneal endothelial cells
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INTRODUCTION

In recent years, there is a continuous rise in the incidence of myopia. According to statistics, the global prevalence of myopia is expected to reach 2.5 billion in 2020[1]. Correcting myopia, especially by refractive surgery, has become the work and research focus on ophthalmologists. Laser-assisted subepithelial keratomileusis (LASEK) was first proposed by Camellin in 1999[2]. It broadened the

indication ranges of refractive surgery, avoided the interference of the negative pressure suction on the vitreous, retina[3], and gradually became a main surgical treatment for refractive surgery. However, in eyes with a preoperative spherical equivalent (SE) greater than 12.00 D, LASEK led to a significant rate of myopic regression associated with corneal subepithelial haze (haze) development[4].

Mitomycin C (MMC) is a commonly used anticancer drug, which can damage DNA structure and function, and inhibit DNA replication and cell proliferation. Recently, MMC has found application in preventing a few complications associated with corneal epithelium in LASEK procedure. Mirza *et al*[5] reported a case of dense and visually significant corneal haze after LASEK and was successfully treated with mitomycin C. Mamjudar *et al*[6] confirmed that MMC could help inhibiting haze formation and restore vision. In addition, MMC was also used to reduce the failure rate of glaucoma filtering operation, prevent recurrence of pterygium, reduce strabismus, adhesions and scar formation after lacrimal surgery, *etc*.[7-10]. However, literature reports that inappropriate use of MMC could decrease the corneal endothelial cell count and functional failure, leading to secondary corneal edema, glaucoma, corneal perforation and other complications. Most complications of MMC were associated with its long-time use, use in high concentration and its effect on corneal limbus[11-12]. Therefore, there are important clinical significances in investigating the safety of MMC during LASEK surgery. In this study, the effects of MMC on the corneal endothelial cells at different exposure time in LASEK surgery was evaluated.

SUBJECTS AND METHODS

Inclusion and Exclusion Criteria Patients who had the subjective will of undergoing LASEK surgery from October 2016 to March 2017 in our hospital were included in this study. For every enrolled patient, the refractive error was required to have been stable for at least 2y and the contact lens wearing time was less than 5y with 2wk of no lens-wearing before the surgery. In addition, the medical examination results of some patients had met the criteria of LASEK, but their first choice was LASIK and existed at least one of these followings: a) Relatively high degree of myopia with thin cornea; b) The occupations with high forward risk of cornea flap, such as armed police, boxing, and athletes, *etc*; c) Special anatomic conditions such as small palpebral fissure or deep socket. These patients agreed to undergo LASEK after comprehending informed consent and were also included in our study. Exclusion criteria: patients with preoperative endothelial cells density (ECD) <2000/mm^2, or the surgery history which affected the corneal endothelial cells such as glaucoma, cataract, and corneal surgery were excluded. All patients had a detailed understanding of the surgical approach, advantages and disadvantages, expected effects, risks and possible complications. All patients signed informed consent, and all procedures performed in studies involving human participants were in accordance with the ethical standards of the General Hospital of Ningxia Medical University Ethics Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Preoperative Examination All the study patients underwent preoperative examinations, which included uncorrected visual acuity (UCVA), best corrected visual acuity (BCVA), dominant eye, autorefraction, anterior segment examnation, fundus examination, intraocular pressure, corneal thickness, axial length, corneal topography and corneal endothelial cell count. The fundus examination was taken by direct ophthalmoscope and three-mirror contact lens after mydriasis.

Surgical Methods Conjunctival sac surface anesthesia was performed with 4% oxybuprocaine, and the epithelium was trephined. A marker was placed on the cornea and centered on the pupil. Then 20% alcohol (0.1 mL) was instilled inside the trephine and left for 15-25s. A cotton swab was used to absorb the alcohol and balanced salt solution (BSS) was copiously instilled to rinse the ocular surface. An intact corneal flap with the diameter of 8 mm and the above as the fundus was separated using the epikeratome from 6 o'clock position and the pedicle was rolled up and accumulated at 12 o'clock position. The laser ablation was performed according to the planned corrected degree after the laser was adjusted and aligned to the pupil center. A new prepared MMC cotton sheet of 6 mm diameter was used to cover the laser ablation area. The operative area was then washed with 20 mL BSS solution. The epithelium flap was relocated and Johnson clear corneal contact lens with the degree of -1.0 D and the diameter of 14.0 mm was placed.

Method of MMC Usage and Categorizing the Patients Ten minutes before the beginning of surgery, a sterile dilute solution of MMC 0.02% was prepared by mixing MMC for injection (2 mg, Zhejiang Haizheng pharmaceutical production) with 10 mL saline. SS-96A Kang, a medical sponge was cut into round sponge with 6 mm diameter and 1 mm thickness under the asepsis procedure and placed in the prepared MMC 0.02% solution to completely soak the solution. The sponge infiltrated with MMC 0.02% was picked up by clamps, with no drop of liquid, and is placed on the central of stroma in cornea ablation area, and the exposure time was according to the diopter: 65-70s for spherical equivalent refraction ≥8.00 D, 50-60s for spherical equivalent refraction was -6.00 D to -7.75 D, 35-45s for spherical equivalent refraction was -3.75 D to -5.75 D, and 15-30s for spherical equivalent refraction≤-3.50 D. The patients were classified into two groups, T1 (15s≤t≤45s) and T2 (45s<t≤70s), based on the time of treatment with MMC 0.02% in the surgery.

Postoperative Management After surgery, anti-inflammatory eye drops (diclofenac sodium; 4 times a day), artificial tears (0.1% sodium hyaluronate eye drops; 5 times a day), and an antibiotic (levofloxacin eye drops; 5 times a day) were administered. The corneal contact lenses were

Table 1 Preoperative and postoperative endothelial cell density with different exposure times mean±SD, cell/mm^2

Groups	Sites	Preoperative	Postoperative			
			1wk	1mo	3mo	6mo
Group T1 (n=98)	Central	2651.14±206.54 (2444.60,2857.68)	2619.03±183.83 (2435.20,2802.86)	2650.54±227.17 (2423.37,2877.71)	2608.81±229.11 (2379.70,2837.92)	2632.50±244.91 (2387.59,2877.41)
	Peripheral	2632.14±238.78 (2393.36,2870.92)	2658.14±234.98 (2423.16,2892.32)	2661.43±206.16 (2455.27,2867.59)	2614.77±223.88 (2380.89,2838.65)	2628.50±234.61 (2393.89,2863.11)
Group T2 (n=98)	Central	2663.12±210.03 (2453.09,2873.15)	2643.47±168.56 (2474.91,2812.03)	2630.78±257.06 (2373.72,2887.84)	2602.44±258.22 (2344.22,2860.66)	2592.37±255.75 (2336.62,2848.12)
	Peripheral	2708.76±270.63 (2438.13,2979.39)	2634.76±242.56 (2392.20,2877.32)	2681.61±281.55 (2400.06,2963.16)	2661.40±302.22 (2359.18,2963.62)	2690.38±244.93 (2445.45,2935.31)

taken out after corneal epithelial healing was over and a steroid (fluorometholone eye drops; 6 times a day) was administered from the fifth day after surgery. The dose of the steroid eye drops was tapered to 1 time per week until withdrawal. Slit-lamp examination was performed a day after surgery to check whether the corneal contact lens were in the position and the healing of corneal epithelium. The patients were followed up at 1wk, 1, 3 and 6mo after surgery. During the follow up, visual acuity, intraocular pressure, refraction and corneal topography were examined and the healing of corneal epithelium and corneal endothelial density were observed.

Evaluation Criteria 1) Safety criteria: intraoperative and postoperative complications including ocular irritation symptoms, healing delay and occurrence of haze[13]; 2) Corneal endothelial cell criteria: cell density (CD) of corneal endothelial cells in central and peripheral cornea, average cell area (ACA), area standard deviation (SD), coefficient of variation (CV, standard deviation/average cell area) and percentage of hexagonal cells (HEX) before surgery and 1wk, 1, 3 and 6mo after surgery. The 4 sites examined were: the site above the eye (12 o'clock position), the site below the eye (6 o'clock position), the site on the nasal side (2 o'clock position of the right eye and 10 o'clock position of the left eye) and the site on the temporal side (10 o'clock position of the right eye and 2 o'clock position of the left eye) around the central site of the cornea. All of the peripheral 4 sites were 3 mm away from the central site of the cornea. All of these sites were measured three times and a clear image was obtained according to the requirements. All the examinations were performed by the same examiner.

Statistical Analysis Data were represented as means ±SD and the statistical analyses have been carried out with SPSS 19.0 software for Windows (SPSS Inc., Chicago, IL, USA). The comparison among groups and comparison of the preoperative and postoperative corneal endothelial cell parameters at different exposure time points were performed by using the repeated measures analysis of variance. P<0.05 was considered statistically significant.

RESULTS

Summary of Patient Data A total of 98 patients (196 eyes) including 50 males and 48 females aged from 18 to 37 years old were included in our study. The participants were divided into Group T1 (98 eyes) and Group T2 (98 eyes). The age and gender between groups were comparable. The refractive error of all patients was stable for more than 2y. The spherical equivalent refraction was −5.81±1.54 D (−1.50 D to −9.25 D). The astigmatic degree was −1.25±1.25 D (0 to −2.50 D), and the corrected visual acuity was 0.9±0.1 (0.8-1.0). The time for contact lens wearing was less than 5y, and time for removing the lens was more than 2wk. The ablation diameters were 6.5 mm in 180 eyes and 6.0 mm in 18 eyes respectively.

Complications The surgical procedure was successful in all study patients with no complications during the surgery. Mild-to-moderate ocular irritation symptom was observed in 13 eyes 1d after the surgery, but the symptoms relieved in 1-2d. Delayed corneal epithelial healing was observed in 4 eyes. They were treated by delaying the removal of corneal contact lens for 2d. According to the Fantes method, the haze was in level 0.5 and level 1 at 1mo after surgery, which recovered 6mo after surgery.

Cell Density of Corneal Endothelial Cells The preoperative and postoperative CD values in Group T1 and T2 at different action time points are shown in Table 1. The central site: Test within group F=1.533, P=0.196 and test between groups F=0.023, P=0.881. The peripheral sites: Test within group F=0.644, P=0.624, and test between groups F=0.670, P=0.415. The results indicated that there was no significant difference of the central and peripheral CD values within a group or between groups at each action time point (P>0.05).

Average Cell Area in Corneal Endothelial Cells The preoperative and postoperative ACA values in Group T1 and T2 at different action time points are shown in Table 2. The central site: Test within group F=0.146, P=0.949 and test between groups F=0.00, P=0.991. The peripheral sites: test within group F=1.48, P=0.216 and test between groups F=0.874, P=0.352. The crossover effects between inter-group and within-group had significant difference (F=4.082, P<0.05). The peripheral ACA values had significant difference between preoperation and 1wk postoperation in Group T1 (P=0.035) and T2 (P=0.015). The results indicated that except the peripheral ACA values increased at

Table 2 Preoperative and postoperative average endothelial cell area with different exposure times mean±SD, cell/mm^2

Groups	Sites	Preoperative	Postoperative			
			1wk	1mo	3mo	6mo
Group T1 (n=98)	Central	375.45±40.19 (335.26,415.64)	383.80±59.73 (324.07,443.53)	377.70±40.78 (336.92,418.48)	387.40±61.16 (326.24,448.56)	378.61±63.89 (314.72,442.50)
	Peripheral	383.92±37.45 (346.47,421.37)	397.60±42.22[a] (355.38,439.82)	378.20±48.24 (329.96,426.44)	391.06±61.30 (329.76,452.36)	390.73±65.02 (325.71,455.75)
Group T2 (n=98)	Central	382.17±42.44 (339.73,424.61)	377.39±42.81 (334.58,420.20)	381.74±42.04 (339.70,423.78)	372.44±49.88 (322.56,422.32)	389.61±55.79 (333.82,445.40)
	Peripheral	371.41±42.41 (329.00,413.82)	395.61±48.97[b] (346.64,444.58)	390.94±70.33 (320.61,461.27)	389.78±45.57 (344.21,435.35)	367.80±51.46 (316.34,419.26)

[a]P=0.035; [b]P=0.015.

Table 3 Preoperative and postoperative coefficient of variation of corneal endothelial cells with different exposure times

mean±SD, cell/mm^2

Groups	Site	Preoperative	Postoperative			
			1wk	1mo	3mo	6mo
Group T1 (n=98)	Central	35.12±7.06 (28.06,42.18)	38.95±6.56[a] (32.39,45.51)	36.16±5.76 (30.40,41.92)	36.64±6.40 (30.24,43.04)	36.86±5.63 (31.23,42.49)
	Peripheral	36.23±6.94 (29.29,43.17)	36.88±6.32 (30.56,43.20)	37.22±6.71 (30.51,43.93)	37.84±7.63 (30.21,45.47)	36.31±6.09 (30.22,42.40)
Group T2 (n=98)	Central	34.68±5.67 (29.01,40.35)	37.86±4.59[b] (33.27,42.45)	35.04±6.06 (28.98,41.10)	36.94±6.95 (29.99,43.89)	36.57±6.51 (30.06,43.08)
	Peripheral	36.29±4.49 (31.80,40.78)	39.72±6.82 (32.90,46.54)	38.08±5.23 (32.85,43.31)	37.71±6.46 (31.25,44.17)	37.23±5.57 (31.66,42.80)

[a]P=0.012, [b]P=0.016.

Table 4 Preoperative and postoperative percentage of hexagon of cells in corneal endothelium with different exposure times (%)

Groups	Site	Preoperative	Postoperative			
			1wk	1mo	3mo	6mo
Group T1 (n=98)	Central	54.15±12.20 (41.95,66.35)	49.35±11.13[a] (38.22,60.48)	51.59±12.66 (38.93,64.25)	49.19±13.11 (36.08,62.3)	50.53±11.90 (38.63,62.43)
	Peripheral	53.43±12.01 (41.42,65.44)	54.88±13.31 (41.57,68.19)	51.26±12.26 (39.00,63.52)	52.24±12.45 (39.79,64.69)	52.46±12.31 (40.15,64.77)
Group T2 (n=98)	Central	55.79±13.10 (42.69,68.89)	48.43±10.93[b] (37.50,59.36)	51.07±12.64 (38.43,63.71)	49.14±14.88 (34.26,64.02)	54.10±11.10 (43.00,65.20)
	Peripheral	53.59±11.83 (41.76,65.42)	52.10±11.59 (40.51,63.69)	51.62±12.47 (39.15,64.09)	50.14±12.88 (37.26,63.02)	51.55±10.90 (40.65,62.45)

[a]P=0.007, [b]P=0.017.

1wk after surgery, there was no significant difference of the central and peripheral ACA values within a group or between groups at other action time points.

Coefficient of Variation of Corneal Endothelial Cells The preoperative and postoperative CV values in Group T1 and T2 at different time points are shown in Table 3. The central site: test within group F=2.281, P=0.065 and test between groups F=1.093, P=0.302. The peripheral sites: test within group F=1.690, P=0.159 and test between groups F=1.008, P=0.318. The significant difference had been showed in crossover effects between inter-group and within-group(F=4.136, P<0.05). The central CV values had significant difference between preoperation and 1wk postoperation in Group T1 (P=0.012) and T2 (P=0.016). The results indicated that there was no significant difference of the central and peripheral CV values within a group or between groups at each action time points, except the central CV values increased at 1wk after surgery.

Percentage of Hexagon of Cells in Corneal Endothelium The preoperative and postoperative HEX values in Group T1 and T2 at different action time points are shown in Table 4. The central site: test within group F=0.058, P=0.811 and test between groups F=0.693, P=0.407. The peripheral sites: test within group F=2.250, P=0.081 and test between groups F=2.04, P=0.157. The crossover effects between inter-group and within-group had significant difference (F=4.165, P<0.05). The peripheral ACA values had significant difference between preoperation and 1wk postoperation in Group T1 (P=0.035) and T2 (P=0.015).

The results indicated that except for the central HEX values which increased 1wk after surgery in group T1 (P=0.007)

Table 5 Preoperative and postoperative standard deviation of endothelial cell area with different exposure times mean±SD

Groups	Site	Preoperative	Postoperative			
			1wk	1mo	3mo	6mo
Group T1 (n=98)	Central	134.65±21.78 (112.87,156.43)	137.18±28.14 (109.04,165.32)	136.03±25.44 (110.59,161.47)	141.08±30.12 (110.96,171.20)	140.42±24.30 (116.12,164.72)
	Peripheral	139.11±32.58 (106.53,171.69)	138.96±27.14 (111.82,166.10)	139.22±25.19 (114.03,164.41)	146.98±32.88 (114.10,179.86)	141.83±33.52 (108.31,175.35)
Group T2 (n=98)	Central	132.62±22.10 (110.52,154.75)	138.83±16.82 (122.01,155.65)	142.76±26.41 (116.35,169.17)	132.55±21.88 (110.67,154.43)	143.93±30.72 (113.21,174.65)
	Peripheral	138.34±24.10 (114.24,162.44)	148.51±25.94 (122.57,174.45)	143.27±22.98 (120.29,166.25)	147.12±31.63 (115.49,178.75)	136.38±25.48 (110.90,161.86)

and T2 (P=0.017), there was no significant difference of the central and peripheral HEX values within a group or between groups at the other action time points.

Standard Deviation of Cell Area in Corneal Endothelial Cells The preoperative and postoperative SD values in Group T1 and T2 at different action time points are shown in Table 5. The central site: test within group F=1.481, P=0.212 and test between groups F=0.006, P=0.937. The peripheral sites: test within group F=1.524, P=0.204 and test between groups F=0.131, P=0.719. The results indicated that there was no significant difference of the central and peripheral SD values within a group or between groups at each action time point (P>0.05).

DISCUSSION

LASEK surgery is mainly carried out in patients with high myopia and relatively thin cornea[14]. However, it can cause a few complications, especially haze. In the past 10y, researchers found that MMC could reduce the incidence of haze and prevent refractive regression[15-16]. MMC is a cytotoxic drug that can inhibit the growth of many types of cells. A previous report suggested that the toxicity of MMC on fibroblasts increased with the prolongation of time[17]. Therefore, it was important to study the influence of MMC on corneal cells including corneal endothelial cells, which keep the relative dehydration state of cornea to maintain the transparency relying their aqueous barrier and pump function. In this study, we compared the effects of MMC on corneal endothelial cell parameters at different application times in order to provide a reference for the rational use of MMC.

In this study, patients were grouped according to the contact time of MMC with the corneal stroma, and corneal endothelial cell parameters were measured. The patients were followed up for 1wk, 1, 3 and 6mo after the surgery. The results showed that CV and HEX of central corneal endothelial cells significantly changed at postoperative 1wk compared with preoperative parameters, which have no difference at postoperative 1mo, suggesting that different action time of MMC could cause transient changes in central and peripheral corneal endothelial cells. This result is similar to the one previously reported by Zhou *et al*[18]. The changes of CV and HEX in a short time period after LASEK surgery were collectively referred to as "acute changes"[19]. It was possibly a kind of reflex stress that chemicals damage corneal stroma layer, which did not significantly affect the barrier and liquid pump function of endothelial cells. Similarly, the acute changes can return to normal, confirming that the recommended MMC action time was reasonable. It is suggested that the MMC should be used cautiously during the surgical process, and its contact time with corneal stroma should be controlled as far as possible. MMC should be used in the central cornea, avoiding contact with the corneal limbus and reducing the impact on the corneal limbal stem cells.

In this study, it was confirmed that there were no significant differences in other corneal endothelial cell indices in other action time points between the two groups, which could eventually return to the preoperative levels. Among all patients, the MMC maximum use time was 70s in one patient whose binocular refractive degree both were −7.75 DS and −1.75 DC×180A. Mild irritation symptoms were observed on the first day after the surgery, which disappeared 5d later with a transparent cornea and well-healed epithelium. In corneal endothelial cells, CD: 2713.80 cells/mm^2, ACA: 368.5 mm^2, CV: 35.9%, HEX: 53%, and SD: 132.2. No other complications were observed. It was speculated that Descemet's membrane could effectively reduce the adverse effects of long time MMC on corneal endothelium. Similarly, the results of previous studies by de Benito-Llopis *et al*[20] and Zare *et al*[21] showed that CD of postoperative central point did not significantly change after MMC treatment. Corneal endothelial cell density is an important index to detect the functional status of corneal endothelial cells and the quantity of corneal functional reserve. The difference might be due to the measuring range, position and counting method of the corneal endothelial cell detecting instrument.

In addition, there was a transient significant increase in ACA of peripheral corneal endothelial cells at 1wk and at 1mo after surgery, the preoperative level was restored. The reason might be that the central corneal endothelium was relatively more affected than peripheral endothelium after the laser ablation area was soaked with 6 mm diameter MMC cotton piece during LASEK surgery, causing the peripheral endothelial cells to migrate towards the center in the form of transient expansion, leading to an increase in the average area of peripheral corneal endothelial cells. However, the number of cases included in

this study was relatively small. Corneal endothelial cell monitoring was not performed within 1wk after surgery, and the follow up time span was long. Due to these limitations, additional studies are required to confirm the outcomes of this study.

To sum up, during LASEK surgery, a single-use of MMC in a reasonable time range does not significantly impact the long-term corneal health status, although transient acute changes in central CV, HEX, and peripheral ACA were observed for a short duration of time after the surgery.

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