·Basic Research·

Influence of transient intraocular pressure elevation during laser *in situ* keratomileusis on rabbit retina thickness

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Abstract

• AIM: To utilize tissue micro measurement to study the effect of transient high intraocular pressure (IOP) induced by different durations of suction during laser *in situ* keratomileusis (LASIK) on rabbit retina thickness.

• METHODS: Sixty healthy New Zealand white rabbits were randomly divided into a control group, and 3 negative-pressure suction groups (20s group, 45s group, and 3min group) and each group was comprised of 15 rabbits (30 eyes); the latter 3 groups were the transient high IOP models. The retinal tissue around the papilledema was separated. Hematoxylin and eosin (HE) staining was carried out to generate slices for light microscopy. The changes in the retina thickness values of each layer were measured for all animals in each group at different postoperative recovery periods and compared with the values recorded for the animals in the control group. The thickness of the retinal tissue showed a normal distribution. The ANOVA was performed by using SPSS13.0 statistic software.

• RESULTS: In the comparison between the 20s and 45s negative-pressure suction groups and the control group, no significant differences were observed, except at 14d. Significant difference was observed between the 3min negative-pressure suction group and the control group, and the retina thickness value of each layer reached a peak at 14d after repair.

• CONCLUSION: Conventional negative suction during LASIK may not lead to significant changes in retinal tissue thickness; however, if the suction duration is increased to 3min, it will cause significant changes in retinal tissue thickness.

• KEYWORDS: laser *in situ* keratomileusis; transient high intraocular pressure; retina thickness

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INTRODUCTION

Laser *in situ* keratomileusis (LASIK) has become the most commonly used method for correcting ametropia because of its advantages, such as broad surgical indication, fast recovery of postoperative visual acuity and light response, and lack of pain [1-3]. However, the safety of this procedure and the potential adverse effects on the ocular structure are concerns. The current basic research on the complications of LASIK has mainly focused on the ocular surface disease [4], and few studies have been conducted to assess the effect of transient high intraocular pressure (IOP) induced by negative-pressure suction during LASIK on the microstructure of the posterior segment of the eye tissue. Chintala [5] suggested that glutamate receptor activation caused by increased IOP promotes retinal ganglion cell apoptosis. We previously found that the negative suction during LASIK has an effect on the retinal structure in a rabbit model [6]. In the clinic, visual changes are commonly used to measure the impact of IOP on the posterior pole, but accumulating evidence suggests that patients have loss of 40% of the retinal nerve fiber layer (RNFL) before the visual field defects manifest [7]. Therefore, changes in retinal thickness are a more sensitive index[8,9] of the damage caused by high IOP, indicating that the effects of LASIK surgery on the retina should be taken seriously. This issue is clinically significant, and it was the motivation for our study. In this study, we assessed the effect of transient high IOP induced by different suction durations during LASIK on rabbit retina thickness by utilizing tissue micro measurements. Our purpose was to provide an experimental confirmation of the safety of LASIK and to present a safe duration for negative-pressure suction.

MATERIALS AND METHODS

In this study, we used 60 healthy rabbits (weight, 2.5-3 kg,

about 5 months old) without eye diseases (provided by the animal laboratory of Inner Mongolia Medical University). Male and female rabbits were included, fed in room temperature. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Inner Mongolia Medical University.

The 60 rabbits were randomly divided into a control group and negative-pressure suction groups (20s group, 45s group, and 3min group) by simple randomization. Each group was comprised of 15 rabbits (30 eyes), which were further divided into 5 observation points by simple randomization: postoperative instant (OD) and 7, 10, 14, 28d after the operation. Three rabbits (6 eyes) of every experimental group are sacrificed in every examination time point. Both eyes of each rabbit were the experimental eyes (operation eye). Suction and laser in LASIK were performed in the eyes of experimental group and only laser ablation was performed in the eyes of the control group. All eyes in the control group and experimental group received antibiotic eye drops after the operation.

Simulating the LASIK operation process, suction of corneoscleral limbus with a suction ring (Moria, Antony, France; IOP>65 mm Hg) was performed in the 20, 45s and 3min negative-pressure suction groups for 20, 45s and 3min, respectively. Phototherapeutic keratectomy (PTK) was performed to cut the corneal epithelium (cutting diameter, 6.0 mm; cutting depth, 50 μm), according to -9.00 D standards. The corneal stroma was scanned by a laser (cutting diameter, 6.0 mm; cutting depth, 127 μm) (Schwind Company, Kleinostheim, Germany), with a laser wavelength of 193 nm, an energy density of 125 mJ•cm^2, and a pulse frequency of 10 HZ. The eyes in the control group were treated only with the laser. Chloromycetin (0.25%) eye drops (Pharmaceutical Co. Ltd., Cangzhou, Hebei Province, China) were administered to all control group and experimental-group eyes after LASIK.

The rabbits in each observation-period group were sacrificed by air embolism at the following points: postoperative instant 0, 7, 10, 14, or 28d. The eyeballs were quickly removed, whereupon the blood was washed away with brine ice, the anterior segment and lens were removed, and the retinal specimens around the papilledema were peeled. The samples were subsequently soaked in 10% formaldehyde fixative for 48h, were dehydrated step-by-step, and were paraffin embedded. Hematoxylin and eosin (HE) staining was carried out to generate slices (ATAGO OPTICAL WORKS, Germany) for light microscopy (Leica Company, Solms, Germany). The changes of retinal thickness in every experimental group were analyzed in different examination time point.

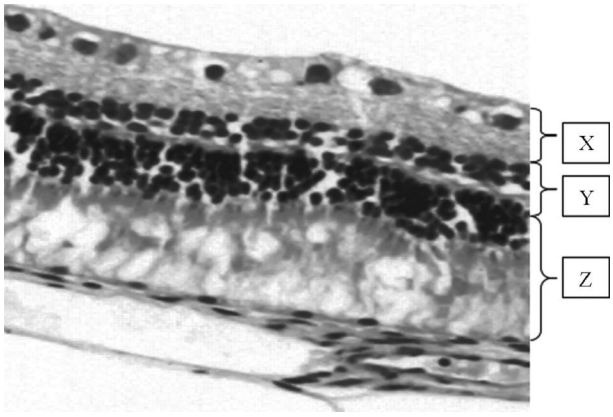

Figure 1 Each layer tissue structure of rabbits´ retina in the control group (HE×400).

The thickness of the retinal tissue showed a normal distribution. The results are expressed as format mean (standard deviation). The ANOVA was performed by using SPSS13.0 statistical software (SPSS Company, Chicago, USA).

RESULTS

The normal retinal structure of the rabbit was divided into 10 layers from the inside to the outside. The quantity of external granular layer cells was the largest (4-7 layers), whereas the quantity of internal granular layer cells was lower (2-3 layers); the quantity of gangliocytes was the lowest. All cells had different sizes and were round or oval, and most of the nuclei were deviated (Figure 1). The total thickness of the retina was measured as X+Y+Z, where X was measured from the retinal inner limiting membrane to the inner plexiform layer (including the RNFL, the ganglion cell layer, and inner plexiform layer), Y was measured from the inner nuclear layer and outer plexiform layer, and Z was measured from the outer nuclear layer, visual cone, and rod layer.

ANOVA was used in our study to compare retinal thickness of each layer at different examination time point between each experimental group and control group, the results are as follows: in the 20s negative-pressure suction group, the retina thickness values of each layer were essentially equal at each of the postoperative repair times (Table 1). No significant differences in the retina thickness values of each layer were found between each different postoperative time group and the control group.

Retinal Tissue Thickness in the 45s Negative-Pressure Suction Group In the 45s negative-pressure suction group, the retina thickness values of each layer varied at different postoperative repair times (Table 2). No significant differences in the retinal tissue thicknesses were found between the postoperative instant 0, 7, 10, 28d and the control group (P=1.000; P=0.545; P=0.053; P=0.220, respectively; P>0.05 for all). However, the retinal tissue thicknesses of the postoperative 14d group and the control group were significantly different (P=0.000, P<0.01). Thus, although

Table 1 The retinal tissue thickness of each layer in negative pressure suction 20s group ($\bar{x} \pm s$, μm)

Time	X (95% CI)	Y (95% CI)	Z (95% CI)	X+Y+Z (95% CI)
Control group	21.73±1.04 (20.89, 22.54)	23.32±0.94 (22.62, 24.10)	71.37±1.62 (70.08, 72.72)	111.92±5.55 (107.91, 116.44)
0d	22.20±0.90 (21.59, 22.95)	24.40±1.53 (23.27, 25.63)	72.42±2.22 (70.44, 73.90)	110.77±6.11 (106.69, 116.33)
7d	21.60±1.26 (20.59, 22.57)	23.51±1.98 (22.13, 24.92)	71.37±2.10 (69.97, 73.04)	112.11±7.20 (106.13, 117.87)
10d	22.74±1.36 (21.72, 23.80)	23.68±0.19 (23.51, 23.81)	72.80±0.90 (72.08, 73.42)	113.57±4.60 (110.46, 117.89)
14d	21.77±0.75 (21.33, 22.50)	24.57±0.14 (24.45, 24.67)	72.21±1.87 (70.91, 73.92)	111.85±0.19 (111.70, 112.00)
28d	21.65±1.33 (20.50, 22.67)	23.68±2.04 (22.32, 25.44)	72.19±1.80 (70.88, 73.64)	112.07±7.23 (107.28, 119.07)
F	1.084	1.537	0.947	0.321
P	0.142	0.208	0.466	0.897

Table 2 The value of retinal tissue thickness of each layer in negative pressure suction 45s group ($\bar{x} \pm s$, μm)

Time	X (95% CI)	Y (95% CI)	Z (95% CI)	X+Y+Z (95% CI)
Control group	21.73±1.04 (20.89, 22.54)	23.32±0.94 (22.62, 24.10)	71.37±1.62 (70.08, 72.72)	111.92±5.55 (107.91, 116.44)
0d	21.80±1.41 (20.57, 22.88)	25.12±0.80 (24.50, 25.71)	72.29±1.96 (70.87, 73.97)	111.24±6.64 (106.72, 116.76)
7d	22.42±0.91 (21.68, 23.04)	26.76±0.33 (26.36, 27.52)	72.84±0.91 (72.06, 73.66)	114.58±2.45 (112.72, 116.71)
10d	23.37±1.02 (22.43, 24.01)	27.00±1.76 (25.86, 28.42)	74.34±1.90 (72.67, 75.82)	116.99±4.82 (113.80, 120.57)
14d[1]	26.98±2.24 (25.23, 28.85)	29.07±1.14 (28.14, 29.84)	78.36±1.34 (77.36, 79.48)	127.95±3.42 (125.40, 130.52)
28d	23.15±1.37 (22.13, 24.30)	25.15±2.17 (23.53, 27.00)	73.09±1.25 (72.09, 74.04)	115.93±3.60 (113.29, 118.40)
F	20.851	23.120	25.993	19.482
P	0.00	0.00	0.00	0.00

[1]There was significant difference between the postoperative14d and experimental control.

Table 3 The value of retinal tissue thickness of each layer in negative pressure suction 3min group ($\bar{x} \pm s$, μm)

Time	X (95% CI)	Y (95% CI)	Z (95% CI)	X+Y+Z (95% CI)
Control group	21.73±1.04 (20.89, 22.54)	23.32±0.94 (22.62, 24.10)	71.37±1.62 (70.08, 72.72)	111.92±5.55 (107.91, 116.44)
0d[1]	31.17±1.81 (29.93, 32.77)	30.68±2.36 (28.84, 32.64)	85.54±5.40 (81.63, 89.79)	135.66±3.05 (133.66, 138.29)
7d[1]	34.33±0.59 (33.83, 34.80)	32.35±3.77 (29.68, 35.88)	88.57±3.72 (85.88, 91.77)	144.41±5.91 (139.88, 148.66)
10d[1]	34.05±3.77 (31.10, 36.71)	36.62±6.44 (32.16, 41.56)	109.65±11.1 (101.43, 118.09)	179.25±13.8 (168.38, 190.90)
14d[1]	39.23±4.03 (36.41, 42.79)	39.23±1.22 (38.41, 40.20)	125.72±13.7 (115.52, 137.07)	191.57±6.52 (186.84, 196.63)
28d[1]	24.58±1.12 (23.63, 25.36)	28.03±0.40 (27.70, 28.30)	77.78±1.10 (76.87, 78.66)	153.93±5.48 (149.81, 158.41)
F	81.191	36.614	80.245	158.666
P	0.00	0.00	0.00	0.00

[1]There was significant difference between the postoperative each group and experimental control.

the retina thicknesses of each layer in the 45s negative-pressure suction group showed some changes as the repair time increased, these values returned to normal at 28d after the operation.

Retinal Tissue Thickness in the 3min Negative-pressure Suction Group In the 3min negative-pressure suction group, the retinal tissue thickness values of each layer varied at different postoperative times (Table 3). The retina thickness values of each layer were increased significantly at the different postoperative times compared to the control group. Namely, compared with the control group, the retina thicknesses of each layer in the 3min negative-pressure suction group significantly increased at different repair times. The peak could be reached in 14d, and the retina was relative thin in 28d.

DISCUSSION

LASIK is most commonly used for the treatment of ametropia. The excimer laser in the LASIK operation is a type of ultra violet light with a wavelength of 193 nm. This laser may generate low thermal-acoustic energy, causing minimum light energy conduction to the retina and vitreous posterior segment; thus, it is not believed to cause retinal and optic nerve damage. Furthermore, our previous work confirmed that this laser treatment does not have an obvious effect on retinal ultrastructure, RNFL thickness, and amino acid levels[10-12]. While the LASIK operation requires a scleral negative suction ring for suckling of the sclera limbus in order to generate a lamellar corneal flap, the transient IOP is greater than 65 mm Hg during the suction process and microkeratome operation, and the duration may increase according to the corneal knife used and the cooperation of the patient. The long-term oppression of the suction ring and a duration increased IOP may cause transient ischemia reperfusion injury of the optic nerve and retina[13]. Tasi *et al* [14] suggested that the retina ganglion cell becomes significantly less 1mo after the LASIK operation. In 2000, a literature has

reported that optic nerve damage appeared in patients after LASIK; the tested negative pressure was 128-141 mm Hg, the IOP was 65 mm Hg, and the negative pressure duration was 45s, which appeared different level of optic nerve ischemia symptom in 1, 3d, and several days after the operation [15]. These changes were accompanied by decreased vision and visual field defect. The possibility that the dramatic rise and fall of IOP caused by negative suction induces changes of the retinal structure and function is a safety issue regarding LASIK, and this has been the concern of clinicians and patients.

The present study suggests several possible mechanisms for the retinal thickness changes caused by transient IOP elevation. The first mechanism is mechanical injury. The scleral lamina cribrosa, which is the junction of unmyelinated nerve fibers and the myelinated nerve fiber, is the weak part of the eye wall. During negative-pressure suction, the shape and volume changes of eyeball and the instantaneous pressure fluctuations may cause optic nerve fiber damage[13]. The second mechanism is abnormal blood flow changes of the retina. The transient high IOP may induce transient ischemia of the retina; when suction is discontinued, the IOP may dramatically drop, the blood flow may instantly resume, and the instantaneous flow change can damage the vascular endothelium and blood cells, thereby resulting in a series of pathological changes[16-18]. The third mechanism is axoplasmic transport disturbance of the optic nerve axons. Animal experiments have confirmed that acute high IOP can lead to axonal transport disorder [13]. Axoplasmic flow is one of the main material transport methods of the visual ganglion cells, and axonal transport dysfunction can lead to a functional obstacle and death of retinal ganglion cells[15,19,20].

This study measured the retina thickness around the papilledema of each layer by using tissue micro measurements, and the results suggested that the simple laser treatment does not affect the retina thickness of each layer. First, no statistically significant difference was found between the 20s negative-pressure suction group and the control group. Second, although the 45s negative-pressure suction group showed some degree of retina thickness change, these changes soon returned to normal compared with the control group; further, these differences were not statistically significant. Third, the retina thickness was significantly greater in the 3min negative-pressure suction group, with the most obvious change at 14d after the operation; however, this group showed a thinning trend over time, indicating that normal recovery was occurring. These results are consistent with those of previous studies[6,10].

In summary, the dramatic rise in IOP during LASIK can cause thickness changes of the retinal tissue. Further, when this negative pressure is prolonged, the damage may become more serious and the changes may become more obvious, possibly resulting in a longer postoperative repair time. Short-term negative-pressure suction (20s or 45s) may not influence or only slightly influence retinal tissue thickness. Although prolonging the suction time to 3min can cause significant changes, the normal state can be restored after a certain repair time; thus, these changes are reversible and will not cause permanent pathological retinal changes. Hence, we propose that the conventional suction of LASIK is safe, and we suggest that performing the operation as promptly as possible can ensure successful completion of the corneal flap while simultaneously shortening the suction time to ensure safety.

Our results are consistent with our expectations prior to conducting the experiments and in agreement with the findings of other studies. This study is basic research, sample is small, and there are some differences between the eyes of rabbits and human beings, so we should expand the sample size to verify the repeatability and accuracy of our research. On the other hand, we will do some research in clinical patients by living examination like optical coherence tomography, reach our final purpose of our series of studies - the safety of LASIK in human beings. The significance of this study lies in applying LASIK surgery to an experimental animal model, discussing the effect of vacuum aspiration on the retina, and observing the factors that influence the safety of the surgery (such as the duration of vacuum aspiration during the surgery). This project has important significance for guiding the design of the surgical procedure for safety, the rigorous screening of patients, and the prophylactic treatment of suspected patients. We will research the mechanisms for the retinal thickness changes caused by transient IOP elevation from the level of molecular and gene. Our final purpose is to provide theoretical basis for the development of safety in LASIK.

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