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Effect of insulin-like growth factor-1 on corneal surface ultrastructure and nerve regeneration of rabbit eyes after laser in situ keratomileusis

Chunyan Wang[1], Yanli Peng[1], Shuling Pan, Li Li*

The Department of Ophthalmology, the Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China

HIGHLIGHTS

- IGF-1 could accelerate the early repair of corneal surface ultrastructure after LASIK.
- IGF-1 could effectively accelerate the corneal nerve regeneration of rabbit eyes after LASIK.
- IGF-1 could relieve the early symptoms of dry eye after LASIK surgery.

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ABSTRACT

To explore the effect of insulin-like growth factor-1 (IGF-1) on corneal surface ultrastructure and nerve regeneration in rabbit models after laser in situ keratomileusis (LASIK). Forty-two healthy New Zealand white rabbits were divided into two groups, the IGF-1 group and the control group, and LASIK surgery was performed. The corneal surface ultrastructure was observed by transmission electron microscopy, and the nerve regeneration was evaluated by counting the newly regenerated nerves at 1 d, 1 w, 2 w, 1 m, 3 m and 6 m after surgery. Dry eye parameters, including the Schirmer *I* test and tear break-up time, were examined at all time points. The examination of corneal ultrastructure showed that the number of corneal epithelial microvilli in the IGF-1 group was significantly higher than that in the normal saline (NS) group except in the second postoperative week ($p < 0.05$). The observation of corneal nerve regeneration showed that the number of regenerated nerve fibers in the IGF-1 group was higher than the control group at all time points ($p < 0.05$). The parameters of dry eye were significantly higher in the IGF-1 group compared to the control group at all time points except at 1 d and 6 m after LASIK. IGF-1 can effectively accelerate the early repair of corneal surface ultrastructure and nerve regeneration after LASIK and relieve dry eye symptoms in rabbit eyes.

1. Introduction

Laser in situ keratomileusis (LASIK) is considered to be a safe and effective operative technique in keratorefractive surgery. With an increasing number of patients undergoing LASIK, the symptoms of dry eye are also increasingly, which can affect patients' post-operative visual quality and overall satisfaction. Dry eye is mostly caused by the large number of corneal nerve fibers that are cut between creating a hinged corneal flap and ablating the stromal bed using an excimer laser. Corneal epithelial cells are damaged during the LASIK procedure [1,2], which results in the reduction of tear secretion and tear film stability. Furthermore, Nagano et al. [3] believes that corneal denervation induces a delay in corneal epithelial wound healing, increasing the incidence of dry eye after LASIK. According to some reports, the repair of corneal nerves after LASIK is a long process. Hence, promoting the early postoperative repair of corneal epithelial cells and nerve injures has important clinical significance. Insulin-like growth factor-1 (IGF-1) is a multi-functional cell factor related to histodifferentiation, proliferation and maturation. It not only can shorten the corneal epithelial wound healing time [3] but also is a non-selective polypeptide neurotrophic factor that can promote the regeneration of peripheral nerves by preventing neuronal apoptosis and promoting axon elongation.

In the present study, rhIGF-1 freeze-dried powder was made into an eye drop for the purpose of observing the effects of IGF-1 on corneal epithelial ultrastructure and nerve regeneration in rabbit eyes after LASIK.

* Corresponding author. Tel.: +86 23 63711526; fax: +86 23 63711527.
E-mail addresses: wangchunyanzeng@126.com (C. Wang), liyanli62@aliyun.com (Y. Peng), zenglingboai@126.com (L. Li).
[1] The co-first authors contributed equally to this work.

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2. Materials and methods

2.1. Preparation for IGF-1

Eye drops were made by diluting 100 μg rhIGF-1 in 100 ml NS to a final concentration of 1 μg/ml; the pH was adjusted to 7.3–7.5 with PBS, and the osmotic pressure was adjusted to 296–305 mOsm/l with normal saline. The left eyes were treated with 1 μg/ml IGF-1 eye drops by 6 times a day after LASIK.

2.2. Animal model of LASIK surgery

Forty-two healthy New Zealand white rabbits weighing 2.0–3.0 kg, both male and female, were purchased from the laboratory animal center of Chongqing Medical University. All rabbits were treated in accordance with the 'Guide for the Care and Use of Laboratory Animals, the Ministry of Science and Technology of the People's Republic of China'. The anterior segment and fundus examinations from the 42 rabbit eyes were normal. We used the eagle EYE-Q excimer laser (Lumenis, USA) and Moria-M2 microkeratome (Moria, France) in the LASIK surgery. LASIK was performed on all 42 rabbits (84 eyes in total). A corneal flap, approximately 110 μm thick, was created with a microkeratome by the hinge located on the nasal side. Then, the corneal stromal bed was exposed for excimer laser ablation within a 6.0 mm optical zone and 60 μm depth. The left eyes received local administration with IGF-1 as the treatment group (IGF-1 group), and the right eyes were treated with normal saline as the control group (NS group).

2.3. Dry eye examination [4]

A Schirmer *I* test was performed without anesthesia. This test was performed by placing a standardized Schirmer test strip in the lateral fornix of the conjunctiva for 5 min and measuring and recording the strip's wetness in millimeters.

The tear film break-up time (TBUT) was measured using fluorescein dye under slit-lamp microscopy. The eyelids of rabbits were opened and fixed by hand to block the spontaneous blinking reflex, and the tear film was observed under a cobalt blue filtered light. The time between the last complete blink and the first appearance of distributed dry spots was recorded.

The dry eye examinations were repeated three times to obtain the average value at the time points 1 d, 1 w, 2 w, 1 m, 3 m and 6 m.

2.4. Transmission electron microscope examination of the ultrastructure

A transmission electron microscope (Hitachi, Japan) was used to observe the ultrastructure. Two rabbits were euthanized with an intravenous overdose of pentobarbital at 1 d, 1 w, 2 w, 1 m, 3 m and 6 m, respectively, after LASIK. The entire cornea was excised and cut into 1 mm × 1 mm pieces, fixed in 4% glutaraldehyde with a 0.2 M phosphate buffer (pH 7.3), post-fixed in 1% osmium tetroxide for 4 h at 4 °C, dehydrated, and embedded in resin. Semi-thin sections (0.5 μm) and ultra-thin sections (60 nm) were prepared and stained with uranyl acetate and lead citrate. All specimens were examined with a transmission electron microscope.

2.5. Golden chloride staining [5] and corneal nerve counting

Five rabbits were sacrificed humanely with an intravenous overdose of pentobarbital at 1 d, 1 w, 2 w, 1 m, 3 m and 6 m after surgery. The corneas were excised and immersed in 10% neutral formalin for 6 h. Then, they were transferred into a 10% formic acid solution for 30 min and transferred to 1% golden chloride until the tissues turned into golden yellow. Finally, the corneas were put into 2% formic acid and washed several times with distilled water. The corneas were cut into slices with a radial pattern and then spread on a microslide. The morphology of the corneal nerves was observed by light microscopy, and the images were captured by computer. In accordance with Rozsa's [6] method, the corneal nerve fibers were counted with light microscopy. Using 10 mm × 10 mm grids in the ocular lens of the microscopy, the number of nerve fibers that passed through the grids was counted. Four visual fields from each sample including both central and peripheral corneas were counted under a 4× objective [5].

Table 1
The number of microvilli of rabbit corneal epithelium of the two groups at different time points after LASIK ($\bar{x} \pm s$).

Time	IGF-1 group	NS group	t	p^*
Pre-op	5.23 ± 0.13	5.12 ± 0.12	1.25	0.258
Post-op 1 d	3.56 ± 0.36	2.85 ± 0.29	3.06	0.022
Post-op 7 d	4.83 ± 0.16	3.99 ± 0.24	5.83	0.001
Post-op 14 d	5.09 ± 0.15[#]	4.86 ± 0.18[&]	1.90	0.106

[#] $p = 0.203$ vs respective pre-op value.
[&] $p = 0.059$ vs respective pre-op value.
[*] $p < 0.05$ means the difference is statistical significance.

2.6. Statistical analysis

The SPSS 17.0 statistical software was used to analyze the data. All data were expressed as means ± SD. The different parameters between the IGF-1 group and the NS group were tested using independent-sample *t*-tests, and a $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Examination of the corneal ultrastructure by transmission electron microscopy (Table 1 and Fig. 1)

The corneal epithelial microvilli arranged neatly before LASIK (Fig. 1A, black arrow). At 1 d after surgery, the corneal microvilli lost their regularity, the stromal cells of cornea arranged sparsely, and a vacuole was observed in the corneal stromal layer in the NS group, while no vacuole was found in the IGF-1 group. The density of corneal epithelial microvilli was significantly decreased in the NS group (Fig. 1B, black arrow), which was less than those in the IGF-1 group (Fig. 1C, black arrow). The difference in the number of corneal microvilli between the two groups was statistically significant ($p = 0.022$). At 1 w after surgery, the number of corneal epithelial microvilli had obviously increased, and the arrangement of microvilli had recovered mostly in the IGF-1 group more than those in the NS group (Fig. 1E and D). There was a statistically significant difference between the two groups ($p = 0.001$). At 2 w after surgery, the number and morphology of corneal epithelial microvilli approached the normal level in the two groups (Fig. 1F and G). The stromal cells returned to their regular form, and no vacuole was found in either groups. There was no statistically significant difference between the two groups at 2 w after surgery ($p = 0.106$). The ultrastructure of rabbit cornea was normal at 1 m, 3 m and 6 m after LASIK surgery.

These results indicated that IGF-1 could accelerate the early repair of the corneal epithelial ultrastructure.

3.2. Dry eye examination (Table 2)

Before LASIK, the mean Schirmer *I* and tear film break up time (TBUT) values were 19.50 mm and 13.93 s respectively. The Schirmer *I* and TBUT values were significantly decreased at 1 d after surgery in the two groups, and there was no statistically significant difference ($p > 0.05$). Comparing the IGF-1 group with the NS group,