DOSE-RELATED DECREASE OF HEMIDESMOSOMES OF THE RABBIT CORNEAL EPITHELIUM AFTER EXCIMER LASER PHOTOREFRACTIVE KERATECTOMY

YUSHI SAKAGAMI, YOSHIAKI NAWA, FUTOSHI TAKETANI and YOSHIAKI HARA
Department of Ophthalmology, Nara Medical University
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Abstract : Currently excimer laser photorefractive keratectomy (PRK) is widely performed to correct refractive errors. It has been recognized that it takes several months for attachment apparatus of the basement membrane of the corneal epithelium to reconstruct their original structure after laser ablation. We investigated dose-related delay of the recovery of hemidesmosomes of the basement membrane of basal cells on the corneal epithelium after PRK in rabbits. Three months after excimer laser ablation, the percentages of hemidesmosomes occupied by basement membrane were measured. The results showed that the percentages significantly decreased as follows: PRK12D group < PRK3Dgroup < control group.

Although only two doses of laser ablation were tested, these results indicated that the more laser ablation the cornea received, the more delay of reconstruction of hemidesmosomes of the basal cells on the corneal epithelium occured.

Key words : excimer laser, PRK, desmosomes

INTRODUCTION

Excimer laser photorefractive keratectomy (PRK) has been widely performed as a keratorefractive surgery since the mid-1980's. Currently, overall satisfaction after PRK for low to severe myopia appears to be very good[1]. Ablated corneas are usually reepithelialized in several days[2]. However, the attachment apparatus of the basement membrane of the epithelium does not recover during the early postoperative course[3]. In the present study, we investigated dose-related delay of reconstruction of hemidesmosomes of the basement membrane of the basal cells on the corneal epithelium after PRK in rabbits.

MATERIAL AND METHODS

Our experimental study was performed according to the declaration of Helsinki. Three-month-old white rabbits were used for the study. Three of four eyes of two rabbits were investigated as control eyes. Due to one eye having mild corneal opacity of unknown cause, it was not included in this study. NIDEK EC-5000 excimer laser was used for the laser surgery. Under general anesthesia with an intramuscular injection of 20-40mg/kg of ketamine hydrochloride, two eyes of one rabbit received three diopters of myopic PRK. Two eyes of one rabbit received twelve diopters of myopic PRK.

Three months after laser ablation, the rabbits were sacrificed by an injection of pentobarbital sodium. The eyes were immediately enucleated. The globes were fixed in Karnovsky's solution at a temperature of 4℃ overnight. Half of the treated corneas were embedded in paraffin, stained with Hematoxilin & Eosin, and observed with light microscopy. With the remaining halves of the corneas, the centers of the corneas were embedded in an epoxy resin. Ultrathin sections were cut with a diamond knife. The basement membrane of the corneal epithelium was observed with a transmission electron microscope. One to six photographs taken with a magnification of ×3300 from each category were analyzed. Prior to analysis the category of each photograph was masked. The photographs were captured into a Macintosh computer (G4) with a scanner (ES-6000, Epson) at a resolution of 800dpi. The images were imported into NIH Image (ver.1.62) as TIFF files.

In each photograph, the total lengths of hemidesmosomes were divided by the total lengths of basement membrane, both of which had been measured five times and averaged. Statistical comparison between each category was done with student's T test.

RESULTS

Light microscopic examination reveals that the corneal epithelium is intact in both the treated groups except for several areas of thickening of the epithelium in the PRK12D group. Details of the light microscopic examinations were previously described[4].

Each transmission electron microscopic photograph has a certain length of the basement membrane. Table 1 shows the results from all photographs measured for the present study. Fig. 1 shows a photograph from the PRK 12D group. The corneas in the PRK 12D group show areas of patchy loss of hemidesmosomes, with areas of infiltration of fibroblasts. The

Table 1. Summary of this study

Category of rabbits	Length of BM (μm)	Length of HD (μm)
control 1	46.2	25.4
control 1	45.6	16.2
control 2	16.7	6.9
control 3	17.6	6.5
control 3	17.6	6.8
control 3	13.4	6.6
PRK 3D 1	38.4	13.2
PRK 3D 1	19.2	5.4
PRK 3D 1	17.6	4.8
PRK 3D 1	18.5	6.0
PRK 3D 2	38.1	11.1
PRK 3D 2	39.9	11.7
PRK 3D 2	18.3	5.6
PRK 12D 1	36.6	8.7
PRK 12D 1	36.0	8.7
PRK 12D 1	18.2	3.6
PRK 12D 1	18.0	5.7
PRK 12D 1	17.7	4.2
PRK 12D 1	17.9	3.9
PRK 12D 2	18.2	4.4
PRK 12D 2	18.2	4.4
PRK 12D 2	18.3	4.5

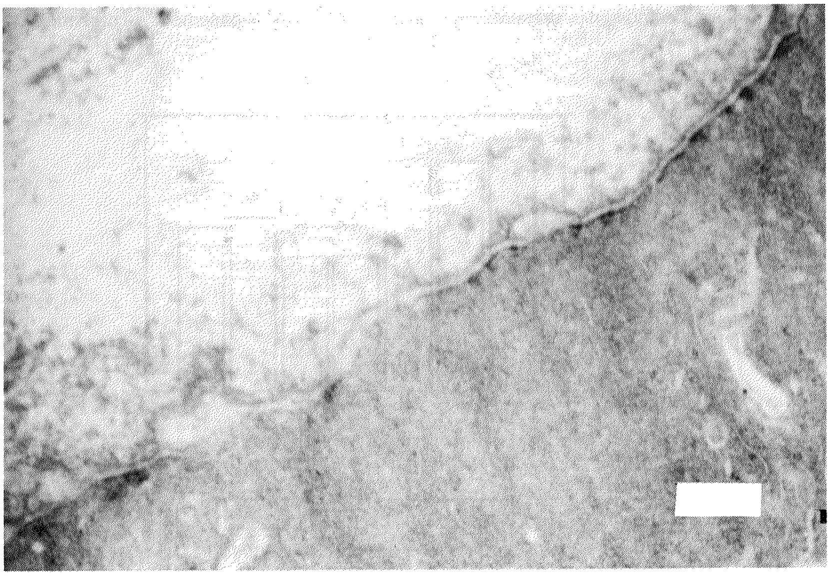


Fig. 1. A photograph from PRK 12D group. The corneal epithelium in the PRK 12D group show areas of pachy loss of hemidesmosomes. Bar=6μm

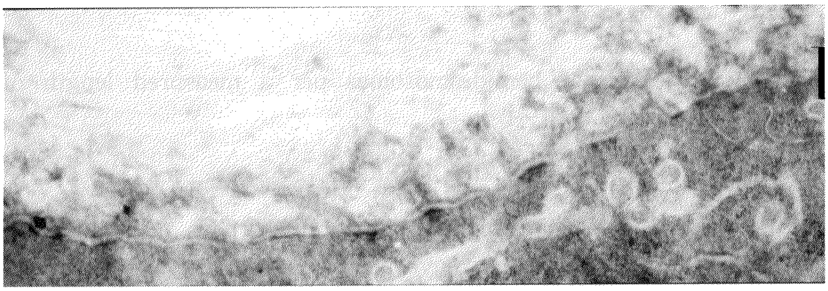
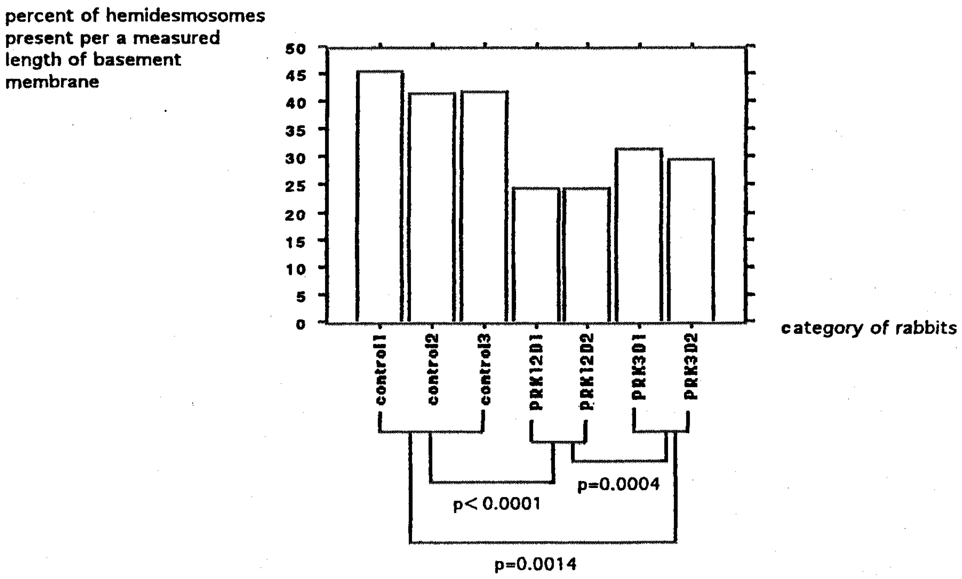


Fig. 2. A photograph from PRK 3D group. The corneal epithelium in the PRK 3D group show occasional areas of loss of hemidesmosomes. Bar=6μm

Fig. 3. A photograph from control group. The corneal epithelium is intact. Bar=3μm

percentage of a total length of hemidesmosomes per a measured length of basement membrane is 23-24%. The corneas of the PRK 3D group appear intact in many areas, with occasional loss of hemidesmosomes and areas of infiltration of fibroblasts (Fig.2). The percentage of a total length of hemidesmosomes per a measured length of basement membrane is 29-32%. The corneas of the control group appear intact (Fig.3). The

Table 2. Percent of hemidesmosomes present per a measured length of basement membrane



percentage of a total length of hemidesmosomes per a measured length of basement membrane is 41-45% (Table 2).

DISCUSSION

Although no Bowman's membrane is present in rabbit corneas, no obvious differences in anchoring fibril structure or distribution have been observed between human corneas and rabbit corneas[5]. In their study, electron micrographs of cross sections and sections parallel to the basement membrane showed that anchoring fibrils inserted into the basal lamina and then splayed out laterally. They were more readily seen in sections taken parallel to the basal lamina, where they were observed to form a complex branching and an anastomosing network below the basal lamina[5]. Therefore the results of the present study may be clinically applicable to human corneas.

In the early postoperative period, the epithelium immediately begins to recover after injury. In guinea pig corneas that received a small (1mm) keratectomy wound, the wound bed was epithelialized completely within 24 hr after wounding[3]. The hemidesmosomes reappeared at 24 hours after injury, and were still incomplete at 7 days after injury[3]. In the rat, epithelial remodeling was complete after 4 weeks. The basement membrane was re-established, and the expression patterns for all the adhesion proteins including laminin-1 and connexin 43 for gap junctions, desmoglein 1 or 2 (desmoglein 1+2) for desmosomes, E-cadherin for adherens junctions, and occuldin for tight junctions, were identical with those characteristics of the intact cornea[6]. In rabbits, the corneal epithelium regained its functional barrier, measured by fluorophotometry, 4 weeks after PRK[7].

After the early postoperative period, the epithelium appears normal except for ultrastructures of the basement membrane. In rabbits, the extent of hemidesmosomes formation was still subnormal 12 weeks after mechanical de-epithelialization by a spatula, with or without PRK[7]. The subepithelial labeling for all the antibodies investigated including integrin subunits alpha 6 and beta 4 (hemidesmosome components) and laminin (a basement membrane component) showed focal discontinuities at the level of the basement membrane in the wound area up to 12 months after PRK[8]. Nakayasu et al investigated rabbit corneas that had undergone myopic PRK three years before with antibody to type Ⅶ collagen and showed that weak reaction was observed in the basement membrane, suggesting incomplete regeneration of anchoring fibrils[9]. In human corneas, the anchoring fibrils and basal lamina do not completely normalize even after 15-16 months[10, 11].

Percentage of basal cell membrane occupied by hemidesmosomes has been used as a marker of recovery of the basement membrane[11, 12]. Azar et al showed that compared with normal corneas (24.5% of the basal cell membrane occupied by desmosomes), two lenticles removed for persistent defects had a marked reduction of hemidesmosomes and basement membrane present under epithelium at 3 and 4 weeks (9.6% of the basal membrane occupied by hemidesmosomes, and 5.4% of the basal cell membrane occupied by hemidesmosomes)[11].

The limitations of our study include a modest number of experimental eyes, and that only two kinds of laser intensity were employed. More data would be needed to deduct this data to a clinical situation.

Our present study showed PRK dose-related delay of reconstruction of hemidesmosomes, which had not been reported previously to our knowledge. Such delay of reconstruction of hemidesmosomes may be related to clinically observed recurrent erosion after PRK[13], which may have been underreported. Our study indicated that the more intense the excimer laser ablation, the more frequently recurrent erosion may occur.

CONCLUSION

The basement membrane of the cornea shows a dose-dependent delay of reconstruction after PRK. An intense excimer laser ablation may cause recurrent erosion of the cornea.

REFERENCES

1) **Brubette, I., Gresset, J., Boivin, J. F., Pop, M., Thompson, P. and Makni, H.** : Functional outcome and satisfaction after photorefractive keratectomy. Part2: survey of 690 patients. Ophthalmology **107** : 1790-1796, 2000.

2) **Stein, R.** : Photorefractive keratectomy. Int. Ophthalmol. Clin. **40** : 35-56, 2000.

3) **Stock, E. L., Kurpakus, M. A., Sambol, B. and Jones, J. C.** : Adhesion complex formation after small keratectomy wounds in the cornea. IOVS **33** : 304-313, 1992.

4) **Nawa, Y.** : An experimental study of the effect of the excimer laser surgery on the histological changes of the cornea. J. Nara Med.Associ. **47** : 145-154, 1996.

5) **Gipson, I. K., Spurr-Michaud, S. J. and Tisdale, A. S.** : Anchoring fibrils form a complex network in human and rabbit cornea. IOVS **28** : 212-220, 1987.

6) **Suzuki, K., Tanaka, T., Enoki, M. and Nishida, T.** : Coordinated reassembly of the basement membrane and junctional proteins during corneal epithelial wound healing. IOVS **41** : 2495-2500, 2000.

7) **Chang, S. W., Hu, F. R. and Hou, P. K.** : Corneal epithelial recovery following photorefractive

keratectomy. Br. J. Ophthalmol. **80** : 663–668, 1996.

8) **Latvala, T., Tervo, K. and Tervo, T.** : Reassembly of alpha 6 beta 4 integrin and laminin in rabbit corneal basement membrane after excimer laser surgery: a 12-month follow-up. CLAO J. **21** : 125–129, 1995.

9) **Nakayasu, K., Watanabe, Y., Gotoh, T., Kato, T., Ishikawa, T. and Kanai A** : Long-term healing of excimer laser ablated rabbit corneas. Nippon Ganka Gakkai Zasshi **103** : 99–107, 1999.

10) **Anderson, J. A., Binder, P. S., Rock, M. E. and Vrabec, M. P.** : Human excimer laser keratectomy. Immunohistochemical analysis of healing. Arch. Ophthalmol. **114** : 54–60, 1996.

11) **Fountain, T. R., de la Cruz, Z., Green, W. R., Stark, W. J. and Azar, D. T.** : Reassembly of corneal epithelial adhesion structures after laser keratectomy in humans. Arch. Ophthalmol. **112** : 967–972, 1994.

12) **Azar, D. T., Spurr-Michaud, S. J., Tisdale, A. S., Moore, M. B. and Gipson, I. K.** : Reassembly of the corneal epithelium adhesion structures following human epikeratoplasty. Arch. Ophthalmol. **109** : 1279–1284, 1991.

13) **Puk, D. E., Probst, L. E. and Holland, E. J.** : Recurrent erosion after photorefractive keratectomy. Cornea **15** : 541–542, 1996. la Cruz, Z., Green, W. R., Stark, W. J. and Azar, D.T.: Reassembly of corneal epithelial adhesion structures after laser keratectomy in humans. Arch. Ophthalmol. **112** : 967–972, 1994.