EXPRESSION OF HEAT-SHOCK PROTEIN 70 IN CORNEAS AFTER LASER IN SITU KERATOMILEUSIS (LASIK)

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Abstract : We investigated the expression of heat shock protein (HSP) 70 in rabbit corneas after laser in situ keratomileusis (LASIK). Pathological examination was performed immediately after LASIK and at one month after the surgery. The superficial corneal stroma was markedly stained with anti-HSP 70 antibody immediately after LASIK. At one month after the surgery the cornea was not positively stained. The clinical relevance of this experimental result is discussed.

Key words : HSP 70, LASIK, cornea

INTRODUCTION

Heat shock proteins (HSPs) are induced when cells are exposed to various kinds of stresses. HSPs have a critical role in the recovery of cells from stress and in cytoprotection, guarding cells from subsequent insults. They protect stressed cells by their ability to recognize nascent polypeptides, unstructured regions of proteins and exposed hydrophobic stretches of amino acids. When doing so, chaperones hold, translocate or refold stress-denatured proteins, and prevent their irreversible aggregation with other proteins in the cell[1].

Nearly all HSPs are constantly expressed under normal growth conditions, where they function to maintain protein homeostasis by regulating protein folding quality control. The chaperone activities of heat shock proteins enable folding of newly synthesized proteins and assist protein translocation across intracellular membranes[2].

HSP70 is one of the most abundantly expressed proteins in humans[3]. HSP70 suppressed ischemia-reperfusion injury, and improved survival after 95% hepatectomy, block insult-induced apoptosis at multiple levels[4]. HSP70 might have immunotherapeutic potential, as HSP70 purified from malignant and virally infected cells can transfer and deliver antigenic peptides to antigen-presenting cells to elicit peptide-specific immunity and, in contrast to its reported pro-inflammatory effects, the administration of recombinant HSP70 can attenuate experimental autoimmune disease[5]. Selective overexpression of HSP70 leads to protection in several different models of nervous system injury[6]. HSP70 may activate natural killer cells[7]. Upregulating HSP70 and causing local necrosis in tumor tissue by hyperthermia offers great potential as a new approach to directly activate the immune system[8].

In normal human corneas, HSP70 is mildly expressed in the epithelium. The stroma and endothelium are not positively stained[9]. In rabbit eyes after photorefractive keratectomy (PRK) with excimer laser, HSP70 was expressed in the superfical stroma[10]. In the cultured

fibroblast, 193nm excimer laser ablation induced transient 8 hours expression of HSP70 mRNA[11].

HSP70 expression after laser in situ keratomileusis (LASIK), currently the most frequently performed keratorefractive surgery, has not been reported to our knowledge. We report the result of an experimental study.

MATERIAL AND METHODS

This experimental study was performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male three-month-old white rabbits (n=6) were used for the study. All LASIK procedures were performed by one surgeon (YN) using a NIDEK MK-2000 microkeratome with the 160-μm thickness plate and the NIDEK EC-5000 excimer laser (NIDEK Co., Gamagori, Japan). Topical anesthesia with bupivacaine 0.5% drops (Benoxil®, Santen Pharmaceutical Co., Osaka, Japan) was instilled. In addition, general anesthesia with an intramuscular injection of 30mg/ kg ketamine hydrochloride (Ketaral®, Sankyo Co., Tokyo, Japan) was performed. The laser was set to create a 6 diopter myopic correction with a 6 mm ablation zone and 1 mm transition zone. The right eyes were treated. The left eyes were used as controls. Immediately after the surgery, three rabbits were sacrificed with an intravenous injection of sodium pentobarbitar (Nembutal®, Dainippon Pharmaceutical Co., Osaka, Japan). One month after the surgery, the other three rabbits were sacrifced in the same manner.

Both eyes were enucleated and the whole globes were immediately fixed in 4% paraformaldehyde (in 0.1M PBS) at 4℃. Thirty minutes after the fixation, the corneas were cut at the limbus and post-fixed overnight. These tissues was dehydrated in a graded series of alcohol and embedded in paraffin. Four-micron-thick slices were cut and deparaffined. The tissue sections were blocked with 10% gout serum and then incubated at room temperature for two hours with a commercially available primary antibody raised against heat shock protein 70 (W27, NeoMarkers, CA, USA) that was diluted 1:100 in PBS; control incubations included incubation with normal mouse IgG (Dako, Kyoto, Japan). The sections were then washed three times for 15 minutes each in PBS containing Triton X-100 (PBS) and incubated at room temperature for 1 hour with the secondary antibody, FITC-conjugated gout anti-mouse IgG (Dako, Kyoto, Japan) diluted 1:500 in PBS. After several washings with PBS, the sections were coverslipped with antifade mounting medium containing propidium iodide (PI, for counter staining with red) and the slides were examined with confocal microscopy (MRC-600®, BioRad, Tokyo, Japan).

RESULTS

The corneas immediately after LASIK had lost their flaps during processing. The superficial stroma showed intense staining for HSP 70 in the central area treated with excimer laser (Fig. 1). Other areas including corneal endothelium, Bowman's membrane, Descemet's membrane, and corneal endothelium did not stain positively for HSP 70. Control corneas did not stain positively for HSP 70. Three corneas in this group showed similar

findings.

The corneas excised at 1 month after LASIK did not stain positively for HSP 70 in any sections examined (Fig. 2). Control corneas did not stain positively for HSP 70. Three corneas in this group showed similar findings.

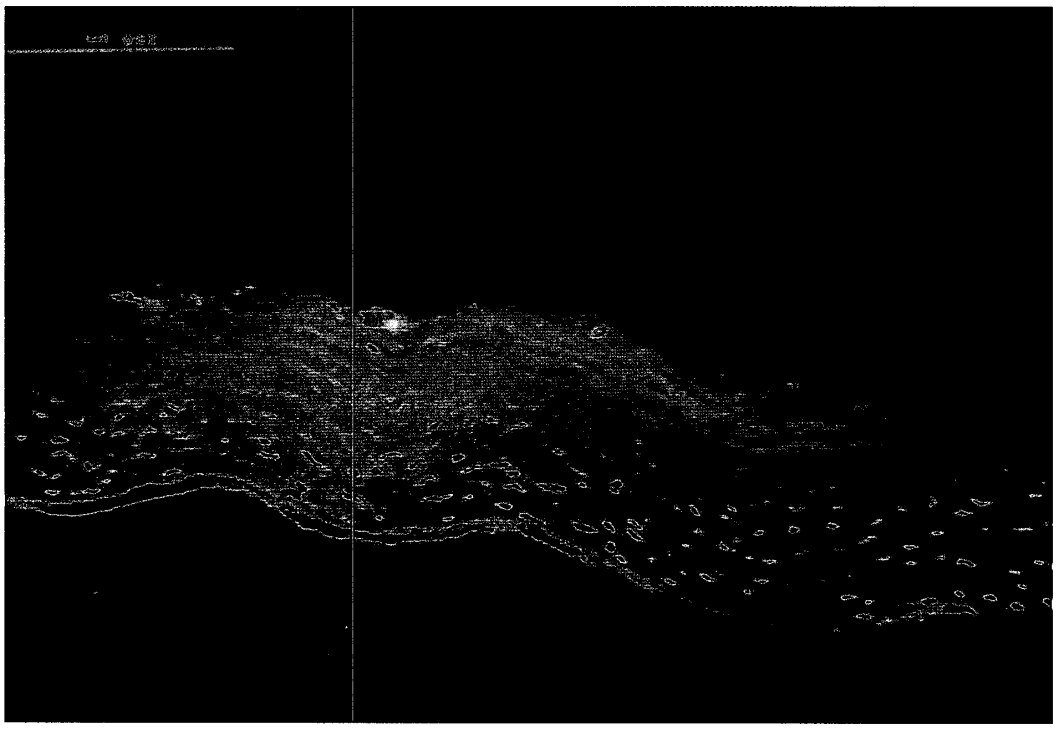

Fig. 1. The cornea immediately after LASIK stained with anti-HSP 70 antibody. The superficial stroma markedly stains positively (green). Bar = 250μm

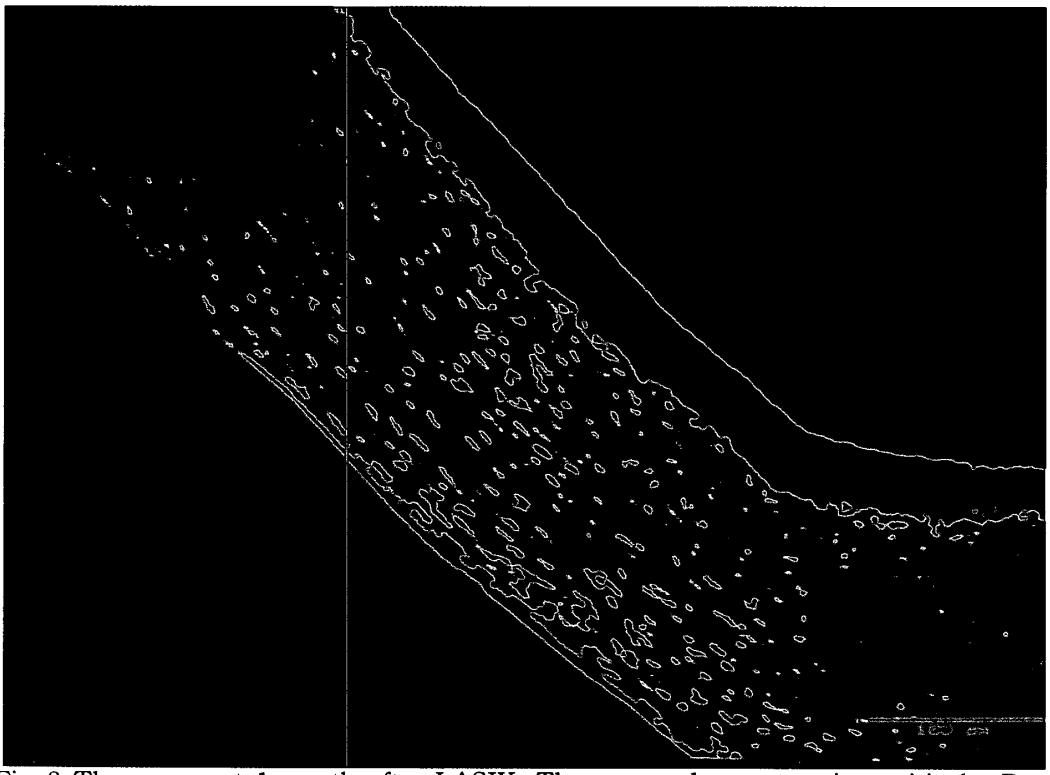

Fig. 2. The cornea at 1 month after LASIK. The cornea does not stain positively. Bar =100μm

DISCUSSION

In the present study it was shown that HSP 70 was markedly expressed in the superficial stroma around the treated area immediately after LASIK. The expression of HSP 70 may result from the insult of the corneal stromal fibrocytes. Excimer laser cut the stromal collagen fiber bundles and cytoplasm of fibrocytes[12]. In addition, it has been reported that tissues abladed with excimer laser undergo temperature rise[13]. This reaction, elevated HSP70 expression, may have been induced in order to protect the cornea from further damage.

In most corneas after PRK various degrees of haze appear at an early stage after surgery and persist for at least several months[14]. During this period the vision deteriorates to various degrees. In contrast, most corneas after LASIK are clear immediately after surgery. The vision recovers quickly[15]. The difference of the time course of vision recovery between PRK and LASIK may stem from the difference of the amount of insult to the corneal epithelium. The epithelium is minimally damaged in LASIK[15]. It was suggested that expression of HSP 70 did not largely affect the corneal haze after PRK, since the corneas after LASIK also expressed marked amounts of HSP 70.

The HSP 70 may act as a molecular chaperone in wound healing of the cornea. In addition, it may act to prevent infection. It has been reported that HSP 70 acts as a block to microbial infection[16]. It has been reported that the incidence of corneal infection is 1/5000 after LASIK[17]. The low incidence rate may be attributed to the expression of HSP 70.

Another possible clinical significance of HSP 70 is that this may cause an allergic reaction. Aberrant production of HSP 70 is reported to be associated with rheumatoid arthritis[18]. In the eye after LASIK, diffuse lamellar keratitis occasionally occurs as a flat diffuse opacity of the superficial cornea a few days after surgery. It usually subsides with the use of steroid eyedrops[19]. Further investigation is needed to clear this association using experimental animals.

The cornea did not stain positively with HSP 70 at 1 month after LASIK. Corneal wound healing continues for several months after excimer laser surgery[19]. It was suggested that HSP 70 did not relate to the chronic wound healing process in post-LASIK eyes.

The disparity of this result with the previous report that HSP 70 was weakly expressed in normal corneas[9] may result from the difference of experimental animals and cross-antigenicity of used antibodies.

In conclusion, HSP 70 was expressed in early post-LASIK rabbit corneas and disappeared at 1 month after LASIK. The clinical significance of this result is a topic for future investigation.

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