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Research article

The effects of proinflammatory cytokines on the apoptosis of corneal endothelial cells following argon laser iridotomy

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ABSTRACT

The aim of this study was to evaluate the relationship between the expression of proinflammatory cytokines and the apoptosis of corneal endothelial cells after argon laser iridotomy (ALI). ALI was performed on each quadrant of the iris in the right eye of mice (ALI1 group). Left eyes were used as control group. The levels of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, and interferon (IFN)-γ in mice eyes were measured, and TUNEL staining was performed 12 h after ALI. Mice in the ALI-Dexa group were pretreated daily with an intraperitoneal injection of dexamethasone for 4 days before undergoing ALI and compared with mice without dexamethasone pretreatment (ALI2 group). Twelve corneas from six rabbits were incubated ex vivo with (n = 6) or without (n = 6) IL-1β. TUNEL staining was performed 24 h after ex vivo incubation. In the mice experiment, the levels of IL-1β, TNF-α, TGF-β, and IFN-γ were increased in the ALI1 group compared to the control group. Although many TUNEL-positive cells were observed in the ALI1 group, those were not detected in the control group. Dexamethasone pretreatment inhibited the increase in the levels of all four proinflammatory cytokines and reduced TUNEL-positive cells. In the rabbit experiment, TUNEL-positive cells were increased in the incubated corneas with IL-1β compared to those without IL-1β. Expression of proinflammatory cytokines following ALI seems to play a role in the apoptosis of corneal endothelial cells after ALI. Dexamethasone pretreatment inhibited increases in proinflammatory cytokines and reduced the apoptosis of corneal endothelial cells.

1. Introduction

Surgical iridectomy and argon laser iridotomy (ALI) have been performed in the treatment and prevention of primary angle closure glaucoma (Go et al., 1984; Pollack, 1980; Quigley, 1981; Ramulu et al., 2007). Surgical iridectomy has been largely replaced by ALI because ALI is a noninvasive procedure easily performed in outpatient clinics (Ramulu et al., 2007). However, several cases of the development of corneal decompensation after ALI have been reported (Schwartz et al., 1988; Wilhelmus, 1992; Zabel et al., 1991). Bullous keratopathy after ALI is one common cause of penetrating keratoplasty in Asian countries (Ang et al., 2007; Shimazaki et al., 2007). Our previous study demonstrated that ALI induces the apoptosis of corneal endothelial cells in rabbit eyes (Youm et al., 2014).

Possible mechanisms of corneal endothelial cell damage following ALI include direct endothelial damage by the laser itself, thermal damage from elevated aqueous humor temperature, iris pigment dispersion, intraocular pressure (IOP) elevations, chronic postsurgical breakdown of the blood-aqueous barrier, and anterior chamber inflammation (Ang et al., 2007; Higashihara et al., 2011; Lim et al., 2006; Schwartz et al., 1988; Smith and Whitted, 1984; Wilhelmus, 1992; Wu et al., 2000). Lim et al. reported that inferior corneal decompensation occurred in 14 subjects after ALI in the superior iris (Lim et al., 2006). This report emphasized the indirect corneal endothelial damage induced by ALI. Higashihara et al. measured the blood-aqueous barrier function in eyes with corneal endothelial decompensation following ALI and showed that

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humoral transport of substances such as prostaglandins and cytokines due to chronic postsurgical breakdown of the blood-aqueous barrier may cause ALI-induced corneal endothelial decompensation (Higashihara et al., 2011). Another study showed that proinflammatory cytokines such as interleukin (IL)-1, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α induce apoptosis of corneal endothelial cells through nitric oxide (NO) (Sagoo et al., 2004). Thus, the aims of this study were to evaluate the expression of proinflammatory cytokines such as IL-1β, TNF-α, transforming growth factor (TGF)-β, and IFN-γ following ALI, and to investigate the relationship between the expression of proinflammatory cytokine and the apoptosis of corneal endothelial cells.

2. Material and methods

Eighty-nine C57/BL6 mice, each weighing 20–25 g, and six New Zealand white rabbits, each weighing 1.8 to 2.3 kg, were used in this study. ALI experiments were performed in mice while corneal tissue cultures were performed in rabbits because mouse corneas were too thin to be removed without any mechanical damage for corneal tissue culture. All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Institutional Review Board approval was obtained from Korea University Guro Hospital, Seoul, South Korea for this study.

2.1. Mouse experiment

2.1.1. Argon laser iridotomy and subgroups

Ketamine hydrochloride (Ketamine HCl, 0.4 mg/10 g body weight; Huons, Seoul, Korea) was injected intramuscularly for general anesthesia. Additional topical anesthesia was administered with 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories, Fort Worth, TX, USA). After general and topical anesthesia, ALI was performed on each quadrant of the iris in the right eye of all mice without using the Abraham iridotomy contact lens (Fig. 1). The same laser setting (spot size, 75 μm; power, 100 mw; duration, 50 ms; count, 32 to 40 times) was maintained throughout this study.

89 mice were divided into four groups: an ALI1 group (the right eyes of 51 mice; n = 51), in which eyes received ALI; a control group (the left eyes of 51 mice used in the ALI1 group; n = 51), in which eyes did not receive ALI; an ALI2 group (the right eyes of 19 mice; n = 19), in which eyes received ALI; and an ALI-Dexa group (the right eyes of 19 mice, n = 19), in which mice received a once-daily intraperitoneal injection of dexamethasone (0.05 mg/10 g of body weight) for 4 days prior to ALI in order to evaluate the effect of systemic steroid pretreatment on the expression of proinflammatory cytokines in eyes and the apoptosis of corneal endothelial cells.

2.1.2. Western blot analysis

The expression of IL-1β, TNF-α, TGF-β, and IFN-γ protein was evaluated by Western blot analysis of the mice eyes. Mice were anesthetized with an intramuscular injection of ketamine hydrochloride and were humanely killed using a chamber filled with carbonic dioxide. Ninety-six eyes of 48 mice in the control (n = 48) and ALI1 (n = 48) groups were enucleated at 1, 12, and 24 h after ALI. Thirty-two eyes of 16 mice were enucleated at each time. Sixteen right eyes of the ALI2 and 16 right eyes of the ALI-Dexa groups were enucleated at 12 h after ALI.

The whole enucleated eyes were lysed and tissue protein was extracted using T-per® tissue protein extraction reagent containing a protease inhibitor mixture (Thermo Scientific, Rockford, IL, USA). The tissue cell extracts were cleared by centrifugation at 13,000 rpm for 10 min at 4 °C, and the supernatants were used for the experiments. The concentration of tissue protein was assessed using a BCA protein assay kit (Thermo Scientific). The proteins in the samples (20 μg) were separated by SDS-PAGE and were transferred to a 0.22-μm nitrocellulose membrane (Whatman Inc., Florham Park, NJ, USA). The nitrocellulose membranes were blocked by 5% nonfat milk for 1 h at room temperature and the membranes were then incubated with primary antibodies overnight at 4 °C. After that the membranes were washed and incubated with secondary antibodies for 1 h at room temperature. The membranes were treated with enhanced chemiluminescence (ECL) reagent (Cat No: 16,021, iNtRON Biotechnology, Inc., Korea) and exposed to ECL film. Densitometric analysis of protein bands detected on ECL film was determined using commercial software (SRX-101A, Minolta Konica, Japan).

2.1.3. Immunohistochemical staining

After euthanasia of 9 mice in the control, ALI1, ALI2, and ALI-Dexa groups, 3 eyes from each group were enucleated 12 h after ALI. Eyes were frozen immediately in optimum cutting temperature (OCT) compound (Tissue-Tek®, Miles Laboratories, Inc., IN, USA) by liquid nitrogen. Frozen tissue blocks were stored at −85 °C until they were sectioned. Central corneal sections (10 μm thick) were cut using a cryostat at −20 °C and were placed on microscope slides (Leica CM 3050 S, Bannockburn, IL, USA). The corneal tissue sections were fixed in cold acetone at −20 °C for 2 min and incubated for 2 h at 37 °C for the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) stain. TUNEL assays were performed using the ApopTag Red In Situ Apoptosis Detection Kit (Chemicon International, CA, USA). 4′,6-diamidino-2- phenylindole nucleic acid stain (DAPI; Sigma Chem. Co., St Louis, MO, USA) was used for

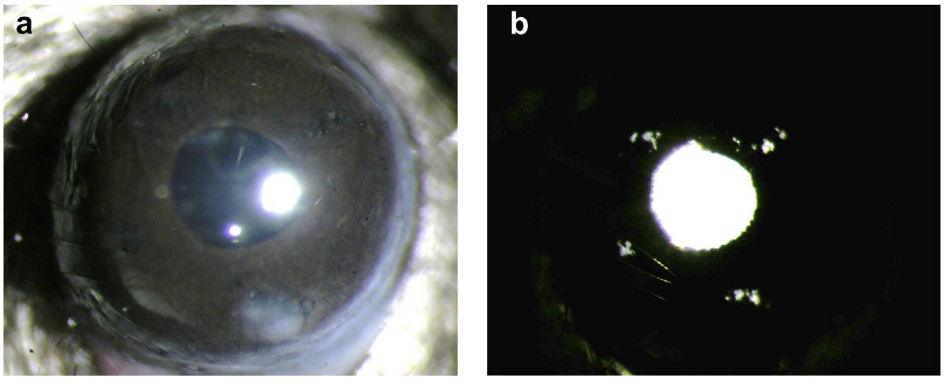

Fig. 1. Anterior segment photographs of a mouse eye after argon laser iridotomy (ALI). (a) ALI was performed on each quadrant of the iris in the right eye of all mice without using an Abraham iridotomy contact lens. (b) Retro-illumination reveals a patent peripheral iridotomy.