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Epithelial cell density in cataractous lenses of patients with diabetes: Association with erythrocyte aldose reductase

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Abstract

In the present study we evaluated the cell density of lens epithelium and its relation to the degree of erythrocyte aldose reductase (AR) in patients with type 2 diabetes. This prospective clinical study included 46 eyes of patients with type 2 diabetes and 48 eyes of patients without diabetes mellitus (DM). Flat preparations of lens epithelial cells (LECs) attached to the anterior capsule were studied. Multiple regression analysis was performed to evaluate the association between lens cell density and age, gender, type of cataract, duration of diabetes, diabetic retinopathy (DR), the levels of glycosylated hemoglobin (HbA1c) and erythrocyte AR. The mean density of LECs of patients with type 2 diabetes was 4141 ± 508 cells/mm^2, which was significantly lower than that of patients without DM (4560 ± 458 cells/mm^2; $p < 0.0001$). Multiple regression analysis revealed that the level of erythrocyte AR was correlated with the reduction of LECs in the eyes of patients with type 2 diabetes. The correlation between the density of LECs and the amount of erythrocyte AR was significant in the diabetic group with a high value of HbA1c (>6.5%) or with DR. These results suggest that the polyol pathway via AR may be associated with the reduction of epithelial cell density in the eyes of patients with DM.

Keywords: aldose reductase; diabetic cataract; diabetes mellitus; lens epithelial cells

1. Introduction

Peripheral lens epithelial cells (LECs) remain capable of proliferation throughout life, elongate and finally differentiate into fiber cells at the equatorial region. The mono-layered LECs in the central region are mitotically quiescent but metabolically active and protect the underlying fiber cells from ultraviolet light and oxidative stress (Li and Spector, 1996; Michael et al., 1998). Nonetheless, cumulative damage and senile changes may reduce the physiological activities of LECs. In fact, previous studies have demonstrated a variety of morphological changes in the epithelium in senile cataract, including vacuolization of the cytoplasm and nuclei, pyknotic nuclei, and signs of degeneration affecting the sizes of cells (Vasavada et al., 1991; Fagerholm and Philipson, 1981). Several investigators have focused on the cell density of lens epithelium in cataractous lenses (Vasavada et al., 1991; Konofsky et al., 1987; Argento and Zarate, 1990). Commonly, a lower cell density has been reported in capsulotomy specimens from lenses with mature and hypermature cataract (Vasavada et al., 1991; Konofsky et al., 1987; Argento and Zarate, 1990).

Animal models of diabetes, such as galactose-fed rats, exhibit intracellular accumulation of sugar alcohol and morphological changes including swelling, rounding of nuclei, and vacuole formation in LECs of cataractous lenses in the eye (Dvornik et al., 1973; Takamura et al., 2003). These morphological distortions are inhibited by aldose reductase (AR) inhibitor (Datiles et al., 1982). AR is localized mainly in the lens epithelium (Akagi et al., 1987), where its activity is approximately 21-fold that in cortical fibers (Hayman and Kinoshita, 1965). Human LECs overexpressing AR are susceptible to osmotic

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and oxidative stress induced by exposure to high glucose concentrations (Kubo et al., 2004). These findings indicate the involvement of AR in cataractogenesis.

Recently we demonstrated a significant association between the level of erythrocyte AR and the severity of cataract in patients with diabetes mellitus (DM) (Oishi et al., 2006). Given the potential significance of these observations, the present prospective clinical study was undertaken to evaluate the relationship between erythrocyte AR level and epithelial cell density in flat preparations obtained from cataractous lenses of diabetic patients.

2. Materials and methods

Forty-six patients with type 2 diabetes (DM group, mean age, 64.0 ± 8.0 years) and 48 patients without DM (control group, mean age, 66.8 ± 7.4 years) who underwent cataract surgery at Fukui University Hospital in Japan from January 2000 to February 2001 were included in this study. None of the eyes had any history of previous ocular surgery. Eyes with ocular complications other than diabetic retinopathy (DR) that may affect cataract formation, such as pseudoexfoliation, severe myopia, glaucoma, uveitis and retinal diseases, were excluded from the study. Preoperatively, the duration of diabetes was examined and the type of lens opacity was estimated in accordance with The Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993). The diagnosis of type 2 diabetes mellitus was defined by a diabetologist at Fukui University based upon the American Diabetes Association (ADA) criteria (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). There was no significant difference in mean age between the DM and control groups. All patients provided informed consent. The research followed the tenets of the Declaration of Helsinki.

Round capsulotomy specimens approximately 5.5 mm in diameter were obtained from patients undergoing cataract surgery using a procedure consisting of curvilinear capsulorrhexis, phacoemulsification, and implantation of an acrylic foldable intraocular lens. The removed LECs with the anterior capsule were immediately fixed in 4% paraformaldehyde dissolved in phosphate-buffered saline (0.1 M PBS, pH 7.4). After dehydration, the capsulotomy specimen was flattened gently on a glass slide, and air-dried for 1 h. It was then stained with hematoxylin and eosin, washed with distilled water, dehydrated through a graded ethanol series, and mounted under a cover glass. Images were acquired using a FUJIX digital camera (HC-2500, Fuji Film) mounted on a light microscope (Olympus). Each specimen was divided into equal quarters, and one field (141,000 μm^2) without mechanical damage was selected randomly from each quadrant, as shown in Fig. 1. The average of the four individual counts per sample was assumed to be the mean density of LECs around the anterior pole. Cell counting and calculation of the cell density of LECs (number of cells/mm^2) were performed automatically using a Mac-SCOPE image analyzer (Mitani Corporation, Japan). Two different examiners, Y.T and Y.K, separately performed the preparation of flat mounts and cell counting, respectively, in a blinded manner. Statistical analysis was performed using Student's *t*-test, Mann–Whitney *U*-test, and simple and multiple regression analysis. Differences at $P < 0.05$ were considered significant. Multiple regression analysis was performed to evaluate the relationship between various factors and the reduction of lens cell density. These factors included patient age, gender, the severity of different types of cataract (cortical, nuclear and posterior subcapsular

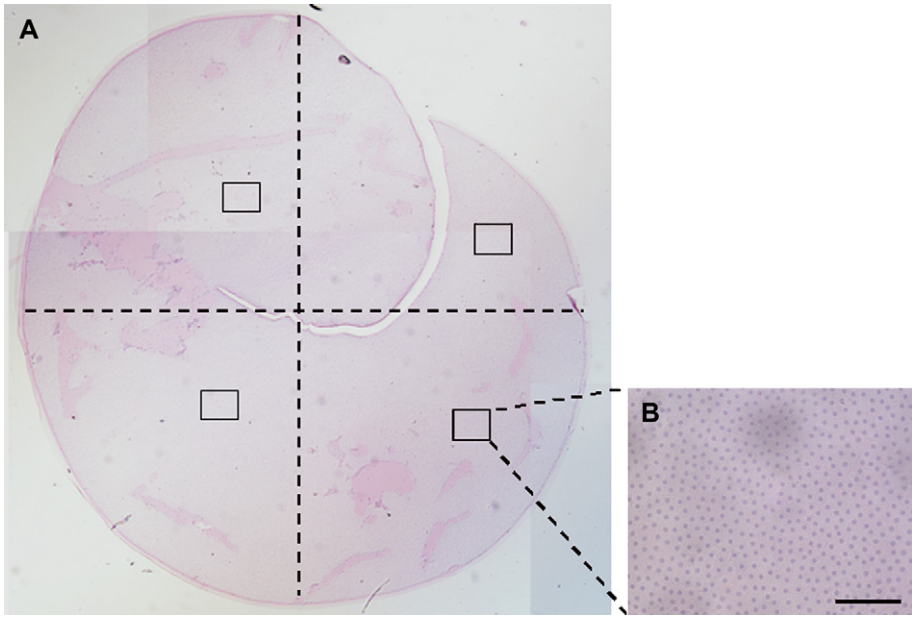

Fig. 1. A. Each capsulotomy specimen was divided into equal quarters by horizontal and vertical lines. One field (141,000 μm^2) without mechanical damage was randomly selected from each quadrant (hematoxylin and eosin). B. At higher magnification, cell counts were performed in each field (hematoxylin and eosin). Bar = 100 μm.