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Aldose reductase expression as a risk factor for cataract

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ABSTRACT

Aldose reductase (AR) is thought to play a role in the pathogenesis of diabetic eye diseases, including cataract and retinopathy. However, not all diabetics develop ocular complications. Paradoxically, some diabetics with poor metabolic control appear to be protected against retinopathy, while others with a history of excellent metabolic control develop severe complications. These observations indicate that one or more risk factors may influence the likelihood that an individual with diabetes will develop cataracts and/or retinopathy. We hypothesize that an elevated level of AR gene expression could confer higher risk for development of diabetic eye disease. To investigate this hypothesis, we examined the onset and severity of diabetes-induced cataract in transgenic mice, designated AR-TG, that were either heterozygous or homozygous for the human AR (AKR1B1) transgene construct. AR-TG mice homozygous for the transgene demonstrated a conditional cataract phenotype, whereby they developed lens vacuoles and cataract-associated structural changes only after induction of experimental diabetes; no such changes were observed in AR-TG heterozygotes or nontransgenic mice with or without experimental diabetes induction. We observed that nondiabetic AR-TG mice did not show lens structural changes even though they had lenticular sorbitol levels almost as high as the diabetic AR-TG lenses that showed early signs of cataract. Over-expression of AR led to increases in the ratio of activated to total levels of extracellular signal-regulated kinase (ERK1/2) and c-Jun N-terminal (JNK1/2), which are known to be involved in cell growth and apoptosis, respectively. After diabetes induction, AR-TG but not WT controls had decreased levels of phosphorylated as well as total ERK1/2 and JNK1/2 compared to their nondiabetic counterparts. These results indicate that high AR expression in the context of hyperglycemia and insulin deficiency may constitute a risk factor that could predispose the lens to disturbances in signaling through the ERK and JNK pathways and thereby alter the balance of cell growth and apoptosis that is critical to lens transparency and homeostasis.

1. Introduction

Over 29.1 million people in the United States and 285 million people worldwide are affected with diabetes mellitus (DM) [1,2]. Nearly all of those with Type 1 diabetes and greater than 60% of those with Type 2 diabetes will have some form of retinopathy in the first decade of being diagnosed [3]. Furthermore, DM is the leading cause of preventable blindness in the United States [4,5]. Each year, there are over 4 million new cases of blindness related to DM [3]. The cost alone in the United States is estimated at over 500 million dollars per year [6]. Increasingly worrisome, diabetes is rapidly growing around the world, making it a significant public health issue [7]. Thus, it is important to investigate the risk factors involved in diabetic eye disease and advance research in medical treatments that delay or inhibit diabetic eye complications.

There are three major eye conditions associated with diabetes: glaucoma, cataracts, and retinopathy. This study will focus on diabetic cataracts. A cataract is an opacification of the lens leading to visual impairment. It affects the ability of the eye to focus light and create clear images [8]. Duration of diabetes and glycemic control are important risk factors in the development of diabetic cataracts [9,10]. Interestingly, diabetic cataracts are more likely to form in those with type 2 diabetes under the age of 18 and in those with type 1 diabetes from ages 18 to 44 years old [11]. There are several

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theories on the pathogenesis of diabetic cataracts, but the activation of the polyol pathway and its enzyme aldose reductase (AR) is of particular interest. In the polyol pathway, AR uses NADPH to reduce glucose into sorbitol. Next, sorbitol dehydrogenase reduces sorbitol into fructose with NAD+ acting as a cofactor [12]. High glucose conditions can activate the polyol pathway causing oxidative stress [13]. There are several ways the polyol pathway induces oxidative stress. First, sorbitol accumulation acts as an osmotic stressor, which leads to the production of reactive oxygen species (ROS) [10]. Second, depletion of NADPH, which is essential to the production of GSH an intracellular antioxidant, causes increased oxidative stress. Third, the production of NADH increases ROS production [10,14]. Finally, fructose can be metabolized into fructose-3-phospate and 3-deoxyglucosone, which are potent non-enzymatic glycation agents. Fructose-3-phospate and 3-deoxyglucosone increase the amount of Advanced Glycation Endproducts (AGEs) leading to ROS generation [14,15]. Furthermore, these oxidative stresses along with hyperglycemia are thought to activate mitogen-activated protein kinases (MAP kinases) which are involved in cell proliferation, survival, and differentiation [16,17]. There are three groups of MAP kinases, which are regulated and activated by phosphorylation: extracellular signal regulated kinases (ERK), c-Jun N-terminal kinases (JNK) and p38 kinases [16]. ERK has two important forms p44 ERK1 and p42 ERK2 (ERK1/2). These kinases are primarily thought of as growth factor signaling kinases that regulate cell proliferation and survival [17,18]. JNK has three forms JNK1, JNK2, and JNK3. JNK is thought to be a stress-activated protein kinase (SAPK) that can activate apoptosis [18]. Since AR is the rate-limiting step in the polyol pathway [19], inhibitors of AR have become an important topic of research.

Preventing diabetic eye disease is complicated. There are many risk factors that contribute to the process as demonstrated by a study finding those with optimal glucose control reduced their risk of developing retinopathy by 76 percent and their progression to retinopathy by 54 percent [20,21]. Even though tight glucose control led to better outcomes in most patients, some patients still developed retinopathy. Thus understanding risk factors that can limit the progression of diabetic eye disease other than glucose control is of fundamental importance. In particular, determining whether specific metabolic pathways and enzymes can be targeted by medical interventions is essential.

Lee et al. [22] previously showed that overexpression of AKR1B1 is associated with cataract development in transgenic mice induced to develop hexosemia from galactose-overfeeding or from experimentally-induced diabetes. Our current study utilized a strain of AKR1B1 transgenic mice we produced using a different promoter construct designed for expression in lens epithelium and outer cortical fiber cells. Our results revealed that AR overexpression in the context of hyperglycemia and insulin dysregulation leads to changes to lens organization as well as significant alterations to ERK and JNK signaling pathways as potentially important elements in the pathogenesis of diabetic cataract.

2. Materials and methods

2.1. Transgenic mice

Transgenic mice designed for lens-specific expression of AKR1B1 were produced by standard methods on a C57BL/6 strain background essentially as described previously [23] with the long term goal of utilizing a human AR (AKR1B1)-expressing mouse model as a screening platform to validate new drug candidates against diabetic cataract formation. The current study was carried out using the strain designated PAR40. AR expression in this stain is controlled by a hybrid α/δ crystallin enhancer/promoter designed to drive transgene expression in lens epithelium and fiber cells in a manner similar to the expression pattern of AKR1B1 in the human lens [24–26]. Transgenic animals were identified by a PCR-based genotyping assay and were backcrossed to generate the transgene in a homozygous state as determined by a quantitative PCR assay using an ABI 7900 qPCR instrument (TaqMan Copy Number Assay and Copy Caller v2.0, Applied Biosystems, Inc. Foster City, CA). Homozygosity of such animals was verified by a backcross to wild type C57BL6 breeders (Jackson Laboratories, Bar Harbor, ME), which resulted in progeny that were verified to carry hemizygous levels of the AR transgene. In all cases, transmission of the transgene followed the expected Mendelian pattern. Unless noted otherwise, all studies with AR-TG mice in this report were carried out with animals homozygous for the AR transgene. PCR genotyping was also used to verify that our AR-TG strain does not carry the *Rd8* mutation of the *Crb1* gene that has been associated with ocular induced phenotypes (data not shown) [27].

Experimental diabetes was induced in AR-TG and nontransgenic mice using a high-dose streptozotocin induction protocol, essentially as described by Graham et al. [28]. Accordingly, AR-TG and C57BL/6 mice, 6 weeks old, were fasted for 4 h, anesthetized by Isoflurane, and injected with streptozotocin (STZ, 160 mg/kg in Na-Citrate, Sigma–Aldrich, MO, USA). Mice were supplied with 10% sucrose water overnight to avoid post-injection hypoglycemia. Blood glucose levels were measured after three days by clipping the tail and using a OneTouch Ultra Mini glucometer (LifeScan, Milpitas, CA). Those with >500 mg/dL blood glucose levels were used for the study. After 16 days of diabetes (or euglycemia in nondiabetic controls), animals were euthanized by CO_2 inhalation and lenses removed from each of four experimental groups: Wild type (WT, C57BL/6), WT with STZ, AR-TG, and AR-TG with STZ (total $n = 12$). A pair of lenses from the same mouse was placed into 300 μL of radioimmunoprecipitation assay buffer (RIPA buffer, Boston BioProducts, MA, USA) and sonicated. Samples were centrifuged at 14,000 rpm at 4 °C for 10 min, and the supernatants (lens cell lysate) were then stored at −20 °C until use.

Expression levels of AKR1B1 in transgenic strains were determined by measuring the rate of DL-glyceraldehyde reduction in the presence of NADPH using lens homogenates in a standard reaction mixture as described previously [29]. Transgene expression levels were also determined by semiquantitative Western blotting as described below.

This research was conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All mice were handled in strict accordance with good animal practice, and all animal work was approved by Institutional Animal Care and Use Committee at The University of Colorado Anschutz Medical Campus (Aurora, CO, USA).

2.2. Sorbitol concentration assay

Lens cell lysates were deproteinized with Deproteinizing Sample Preparation Kit (BioVision, Milpitas, CA). Sorbitol in neutralized samples was measured using a D-Sorbitol Colorimetric Assay Kit as described by the manufacturer (BioVision).

2.3. Protein concentration assay

Protein concentration of lens cell lysate samples were determined using BCA (bicinchoninic acid) Protein Assay Reagent (Thermo Scientific, IL, USA).

2.4. Western blotting

Lens cell lysate samples were mixed with Laemmli buffer (Sigma–Aldrich) and were heated to 95 °C for 10 min. After resolu-