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Aldose reductase inhibition alleviates hyperglycemic effects on human retinal pigment epithelial cells

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ABSTRACT

Chronic hyperglycemia is an important risk factor involved in the onset and progression of diabetic retinopathy (DR). Among other effectors, aldose reductase (AR) has been linked to the pathogenesis of this degenerative disease. The purpose of this study was to investigate whether the novel AR inhibitor, beta-glucogallin (BGG), can offer protection against various hyperglycemia-induced abnormalities in human adult retinal pigment epithelial (ARPE-19) cells. AR is an enzyme that contributes to cellular stress by production of reactive oxygen species (ROS) under high glucose conditions. A marked decrease in cell viability (from 100% to 78%) following long-term exposure (4 days) of RPE cells to high glucose (HG) was largely prevented by siRNA-mediated knockdown of AR gene expression (from 79% to 97%) or inhibition using sorbinil (from 66% to 86%). In HG, BGG decreased sorbitol accumulation (44%), ROS production (27%) as well as ER stress (22%). Additionally, we demonstrated that BGG prevented loss of mitochondrial membrane potential (MMP) under HG exposure. We also showed that AR inhibitor pretreatment reduced retinal microglia-induced apoptosis in APRE-19 cells. These results suggest that BGG may be useful as a therapeutic agent against retinal degeneration in the diabetic eye by preventing RPE cell death.

1. Introduction

In 2010, an estimated 227–285 million people in the world had diabetes mellitus and its associated chronic hyperglycemia and secondary complications [1]. Hyperglycemia or high blood glucose (126 mg/dl when fasting or 200 mg/dl 2 h after meals) may cause damage to peripheral nerves as well as to cells of the renal and cardiovascular systems [2–4]. Diabetes is associated with increased risk for glaucoma, cataract, and retinopathy and is considered the leading cause of blindness among adults [5]. Long-term diabetes leads to retinal edema [6–8] and increased apoptosis in a range of retinal cells including pigment epithelial cells, endothelial cells and pericytes [9–11]. Among the diabetic stressors, reactive oxygen species (ROS), endoplasmic reticulum (ER) stress as well as mitochondrial fragmentation are thought as the important causes of apoptosis [12–16]. Trudeau and colleagues have hypothesized that high glucose (HG) triggers mitochondrial morphological change and cytochrome c release in retinal endothelial cells (RECs) as well as retinal pericytes, further potentiating cell apoptosis [10,11,17], even leading to DR [18]. Extensive investigation on the role of ER stress in hyperglycemia has been shown that high glucose is linked to the unfolded protein response (UPR), production of ROS, and increased apoptosis [14].

Aldose reductase (AR), a member of aldo–keto reductase super family, catalyzes the conversion of glucose to sorbitol as the first step in the polyol pathway [19]. In a variety of diabetic target tissues, AR is linked to pro-inflammatory responses [20,21]. In the eye, AR is linked to diabetic complications such as cataract and posterior capsule opacification (PCO) [22,23]. Additionally, experimental studies demonstrated that genetic or pharmacological blockade of AR prevents the onset and progression of many of the retinal sequelae of diabetes including pericyte loss [24], capillary degeneration [21] and increased markers of oxidative stress [25]. As byproducts of long-term hyperglycemia, advanced glycation endproducts (AGEs) accumulate in the diabetic retina and

Abbreviations: AGEs, advanced glycation endproducts; AR, aldose reductase; ARI, aldose reductase inhibitor; BGG, beta-glucogallin; DR, diabetic retinopathy; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; HG, high glucose; MAPK, mitogen-activated protein kinase; MMP, mitochondrial membrane potential; PCO, posterior capsule opacification; PBMCs, peripheral blood mononuclear cells; RECs, retinal endothelial cells; RMG, retinal microglia ; ROS, reactive oxygen species; RPE, retinal pigment epithelium; UPR, unfolded protein response.

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induce expression of inflammatory molecules in a variety of cell populations including retinal microglia (RMG) [26]. Our previous studies showed that blockade of AR by AR inhibitors (ARIs) or genetic ablation alleviated ocular inflammatory responses such as cytokines secretion and ROS production as well as cell migration [27,28]. Therefore, AR may be involved in AGE-induced ocular inflammation in diabetic patients. Additionally, HG triggers up-regulation of AR in human retinal pigment epithelial (hRPE) cells and peripheral blood mononuclear cells (PBMCs) [29,30], which may enhance glucose metabolism through the polyol pathway and increased production of ROS.

β-Glucogallin (BGG), a novel ARI isolated from Indian gooseberry (*Emblica officinalis*) [31], inhibits AR activity in cells and ocular tissues [27,32]. Lens culture in HG condition demonstrated the inhibitory activity of BGG as evidenced by decreased accumulation of sorbitol [31], an inducer of osmotic stress associated with diabetic cataract development [33]. BGG has low cytotoxicity and is capable of reducing ROS production and mitogen-activated protein kinase (MAPK) activation triggered by endotoxin [27]. However, the effect of BGG on preventing of hyperglycemia-induced stresses in ocular cells is still unknown. To determine whether BGG holds promise as an effective agent in prevention of hyperglycemic defects, we conducted studies using BGG in RPE with HG conditions. We explored the efficacy of BGG in alleviation of HG-induced cell growth inhibition, ROS production and ER responses as well as mitochondrial damage in adult retinal pigment epithelial (ARPE-19) cells. We further probed the ability of ARI treatment in activated RMG to prevent apoptosis of ARPE-19 in a co-culture model. Our results demonstrate the role of AR in hyperglycemia and suggest that BGG is a potent natural agent to prevent some of the ocular complications of diabetes.

2. Materials and methods

2.1. Materials and cell culture

BGG was obtained as previously reported [32]. Sorbinil was generously provided by Pfizer Central Research (Groton, CT, USA). Adult retinal pigment epithelium (ARPE-19) cells were purchased from ATCC (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM; Corning Cellgro, Manassas, VA, USA) containing low glucose (1 g/L) and supplemented with 4 mM L-glutamine, 10% (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained in a humidified incubator containing 5% carbon dioxide at 37 °C.

2.2. Cell viability assay

The effect of high glucose (30 mM) on cell viability was determined by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) (Sigma–Aldrich, St. Louis, MO, USA) as described [34]. Briefly, ARPE-19 cells (10^5) were seeded into individual wells of a 24-well tissue culture plate and were incubated overnight at 37 °C, 5% CO_2. Cells were then treated with BGG under low or high glucose for either 2 or 4 days. After treatment with indicated time, 50 μl MTT (5 mg/ml in PBS) was combined with 450 μl of culture medium from each well and incubated for 4 h. Levels of MTT formazan reaction product were determined by measuring the absorbance at 570 nm by using BioTek Synergy™ 4 Hybrid Microplate Reader.

2.3. Small interfering RNA (siRNA) transfection

Control siRNA and AKR1B1 (AR) siRNA were purchased from Qiagen (Valencia, CA, USA). Transient transfection of siRNA was performed using HiPerFect transfection reagent (Qiagen) according to the manufacturer's protocol. HLE B3 cells (10^6 cells) were seeded in a 100 mm culture dish. After 16 h cells were ~70% confluent and cells were transfected with control or AR siRNA (10 nM) and cultured for an additional 72 h. Efficiency of AR knockdown was confirmed by Western blot.

2.4. Western blotting

Lysates were prepared by suspending cells in Laemmli sample buffer (Sigma–Aldrich) and heated to 100 °C for 10 min, and resolved by SDS–PAGE (Bio-Rad, Hercules, CA, USA). Proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and primary antibodies were used for immunodetection: rabbit anti-AR (1:1000) [28] or mouse anti-actin (1:4000, Sigma–Aldrich) or rabbit anti-p-JNK, JNK, p-ERK, ERK, p-p38, p38 and Bip/GRP78 (1:1000, Cell signaling Technology, Inc., Danvers, MA, USA). Secondary anti-mouse and anti-rabbit antibodies conjugated to horseradish peroxidase (1:5000, Millipore, Bedford, MA, USA), as well as the Western Blot Substrate kit (Bio-Rad) were used to detect chemiluminescence using a BioRad ChemiDoc™ XRS+ imaging system.

2.5. Sorbitol colorimetric assay

ARPE-19 cells (10^6 cells) were incubated in 100 mm dish. After treatment with BGG and low or high glucose, cells were collected and washed with cold PBS twice. The cell lysates were followed by deproteinization with Deproteinizing Sample Preparation Kit (BioVision, Milpitas, CA, USA). Sorbitol detection with neutralized samples was performing using a D-Sorbitol Colorimetric Assay Kit and protocol (BioVision).

2.6. Detection of ROS levels

ARPE-19 cells (5×10^3 cells) were incubated in a 96-well plate and treated with BGG and low or high glucose followed by incubation with ROS-sensitive dye fluorophore 2′,7′-dichlorofluorescencein diacetate (Sigma–Aldrich) for 30 min. The fluorescence was measured with a BioTek Synergy™ 4 Hybrid Microplate Reader at excitation of 485 nm and emission of 528 nm.

2.7. Detection of mitochondrial membrane potential

This method was followed as previous described [34]. To determine MMP, the detached ARPE-19 cells were stained with 100 nM MitoTracker Red CMXRos (Invitrogen, Carlsbad, CA, USA) in DMEM medium for 20 min at 37 °C in the dark. After trypsinization, the stained cells were analyzed by fluorescence-activated cell sorting (FACS). All data was evaluated using Cell Quest software (BD Biosciences, San Jose, CA, USA).

2.8. Apoptosis of ARPE-19 cells

Primary mouse RMG were obtained as previous described [28]. RMG were first activated by LPS (Sigma–Aldrich) exposure for 6 h. Activated RMG were seeded in the upper chamber of a transwell device and ARPE-19 cells were seeded in the bottom chamber. After co-culture for 2 days, apoptosis of RPE cells was measured using an Annexin-V-FITC Apoptosis Detection kit (BD Biosciences) as described [34]. Apoptotic cells were analyzed by FACS. All data was evaluated using Cell Quest software (BD Biosciences).

2.9. Statistical analysis

Results are shown as the means ± SEM of at least three experiments. Data were analyzed by Student's *t* test with *P* value of <0.05 considered significant.