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Beta-glucogallin reduces the expression of lipopolysaccharide-induced inflammatory markers by inhibition of aldose reductase in murine macrophages and ocular tissues

Kun-Che Chang [a,b], Brian Laffin [a], Jessica Ponder [b], Anna Énzsöly [c], János Németh [c], Daniel V. LaBarbera [b], J. Mark Petrash [a,b,*]

[a] Department of Ophthalmology, School of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, CO 80045, USA
[b] Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Science, University of Colorado, Anschutz Medical Campus, Aurora, CO 80045, USA
[c] Department of Ophthalmology, Semmelweis University, Budapest, Hungary

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ABSTRACT

Aldose reductase (AR) catalyzes the reduction of toxic lipid aldehydes to their alcohol products and mediates inflammatory signals triggered by lipopolysaccharide (LPS). Beta-glucogallin (BGG), a recently described AR inhibitor, was purified from extracts of the Indian gooseberry (*Emblica officinalis*). In this study, we found that BGG showed low cytotoxicity in Raw264.7 murine macrophages and effectively inhibited AR activity as measured by a decrease in sorbitol accumulation. In addition, BGG-mediated inhibition of AR prevented LPS-induced activation of JNK and p38 and lowered ROS levels, which could inhibit LPS-induced apoptosis. Uveitis is a disease of the eye associated with chronic inflammation. In this study, we also demonstrated that treatment with BGG decreased the number of inflammatory cells that infiltrate the ocular media of mice with experimental uveitis. Accordingly, these results suggest BGG is a potential therapy for inflammatory diseases.

1. Introduction

The Indian gooseberry (*Emblica officinalis*), commonly known as Amla, is used in the practice of Indian traditional medicine (Ayurveda) to minimize the effects of diabetes and its complications. We previously showed that treatment of experimentally diabetic rats with crude extracts from Amla fruit delayed the onset and progression of cataracts and prevented the accumulation of sorbitol and diabetes-induced markers of lipid peroxidation and protein oxidation products in the lens [1,2]. Because these results were consistent with the effects of aldose reductase (AR) inhibition, we used a bioassay-guided scheme to search for putative AR inhibitors (ARI), using human AR (AKR1B1) activity as an assay read out. Fractionation of materials in an Amla extract resolved several compounds with ARI activity. Structure elucidation by ^{1}H NMR identified the major inhibitor as 1-*O*-galloyl-beta-D-glucose, also known as Beta-glucogallin (BGG) [3]. Inhibition studies demonstrated an IC_{50} of approximately 17 μM against AKR1B1, and virtually no inhibition when assayed under similar conditions with the other major human AKR1 family members AKR1B10 (small intestine reductase, HSIR) and AKR1A1 (aldehyde reductase). In organ culture assays of lenses dissected from AKR1B1 transgenic mice, BGG decreased sorbitol accumulation by about 75% and protected against the loss of glutathione [3]. Ramana and others have shown that AR inhibition by sorbinil and a variety of other validated ARIs lowered the levels of inflammatory markers associated with exposure to lipopolysaccharide (LPS) endotoxins [4–6]. To determine whether BGG holds promise as an anti-inflammatory agent, we conducted studies to measure its toxicity toward mammalian cells in a tissue culture setting. Studies have also been carried out to explore the efficacy of BGG in prevention of lymphocyte infiltration into the anterior and posterior compartments of the mouse eye in the endotoxin-induced uveitis model. We further probed the ability of BGG to reduce the expression of some pro-inflammatory markers in macrophages following exposure to endotoxin. Our results demonstrate consistency with other validated ARIs and confirm that it has favorable bioavailability in the intact animal. The results of our studies substantiate the case for BGG as a potent and effective natural anti-inflammatory agent.

Abbreviations: AR, aldose reductase; ARI, aldose reductase inhibitor; LPS, lipopolysaccharide; BGG, beta-glucogallin; ROS, reactive oxygen species; DR, diabetic retinopathy.

* Corresponding author at: Department of Ophthalmology, School of Medicine, University of Colorado Anschutz Medical Campus, 12800 East 19th Avenue, Mail Stop 8311, RC1-North, 5100, Aurora, CO 80045, USA. Tel.: +1 303 724 0681; fax: +1 303 848 5014.

E-mail address: mark.petrash@ucdenver.edu (J.M. Petrash).

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2. Materials and methods

2.1. Materials and cell culture

BGG was obtained and purified as previously reported [3]. LPS (*Salmonella enterica* serotype typhimurium) was purchased from Sigma–Aldrich (St. Louis, MO). Sorbinil ((4S)-6-Fluoro-2,3-dihydro-spiro[4H-1-benzopyran-4,4′-imidazolidine]-2′,5′-dione) was generously provided by Pfizer Central Research (Groton, CT). Raw264.7 murine macrophages were cultured in complete Dulbecco's Modified Eagle Medium 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

Cells (10^4 cells) were incubated in a 96-well plate and treated with Sorbinil, H_2O_2 and the indicated concentration of BGG. After 24 h treatment, the cell viability was determined by LIVE/DEAD® viability/cytotoxicity Kit (Invitrogen, Carlsbad, CA) as previously described [7]. Live cells were quantified by determining the Calcein fluorescence (F_{528}) collected at 528 ± 20 nm from excitation at 485 ± 20 nm using a BioTek Synergy™ 4 Hybrid Microplate Reader (Bio Tek, Winooski, VT). Fluorescent data were analyzed as outlined in the manufacturer's instructions.

2.3. Sorbitol colorimetric assay

Cells (10^7 cells) were incubated in T75 flasks. After treatment with ARIs and LPS, cells were collected and washed with cold PBS twice. The 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).

2.4. Western blotting

Lysates were prepared by suspending cells in Laemmli sample buffer (Sigma–Aldrich) and heating to 100 °C for 10 min. After a brief centrifugation, materials were resolved by SDS–PAGE (Bio-Rad, Hercules, CA) and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). The following primary antibodies were used for immunodetection: rabbit anti-mouse p-JNK, p-p38, JNK and p38 (Cell Signaling Technology, Inc., Danvers, MA). Secondary antibodies conjugated to horseradish peroxidase (Millipore, Bedford, MA) and the Western Blot Substrate kit (Bio-Rad Laboratories) were used to detect chemiluminescence using a BioRad ChemiDoc™ XRS + imaging system.

2.5. Detection of ROS levels

Cells (10^4 cells) were incubated in a 96-well plate without or with Sorbinil or BGG and treated with the ROS-sensitive dye fluorophore 2′,7′-dichlorofluorescein diacetate (Sigma) for 30 min. Subsequently, the cells were exposed to LPS for 60 min and the fluorescence was measured with a BioTek Synergy™ 4 Hybrid Microplate Reader at excitation of 485 nm and emission of 528 nm.

2.6. Uveitis

Experimental uveitis was induced in male C57BL/6 mice with a single intraperitoneal injection of lipopolysaccharide, essentially as described by Tuo et al. [8]. Animals were randomly assigned to different treatment groups (in all cases, $N = 3$) including a control group receiving PBS, and sorbinil and BGG groups treated at 10 mg/kg. Treatments were carried out by intraperitoneal injections of 0.1 ml solution immediately after the LPS treatment. Mice were euthanized 24 h later and eyes fixed in formalin and embedded with paraffin. Tissue slices were mounted on coded glass slides, stained with hematoxylin–eosin and used to manually count inflammatory cells in the vitreous cavity and anterior chamber. Cells were counted with the observer masked to the treatment group.

2.7. 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.

3. Results

3.1. BGG exhibits low cytotoxicity in Raw264.7 murine macrophages

Although BGG has been implicated as a therapeutic agent against diabetic cataract [3], its cytotoxicity has not been assessed. In this study, we examined the cytotoxicity of BGG in Raw264.7 murine macrophages. After 24 h treatment, we found there is virtually no cytotoxicity with BGG up to a concentration of 50 μM, whereas some slight toxicity was apparent when cells were incubated in the presence of 100 μM BGG (84 ± 1.6% survival). In contrast, 200 μM H_2O_2 (positive control) shows 50% cytotoxicity (Fig. 1). The relatively low cytotoxicity of BGG suggests it may be tolerated over a broad range of doses.

3.2. Aldose reductase inhibitors decrease sorbitol levels in macrophages

To determine the AR inhibition efficiency in macrophages, we measured the amount of sorbitol, which is produced by AR from glucose, after treatment of cells with BGG. For comparison, we also measured sorbitol levels in cells incubated with Sorbinil, a well-characterized ARI shown previously to diminish sorbitol accumulation in cell culture models and animal tissues [3,9]. We found that Sorbinil or BGG treatment decreased sorbitol levels by 44% or 40% compared to vehicle in macrophages without LPS exposure, respectively (Fig. 2). In addition, LPS induced a 13% increase in sorbitol

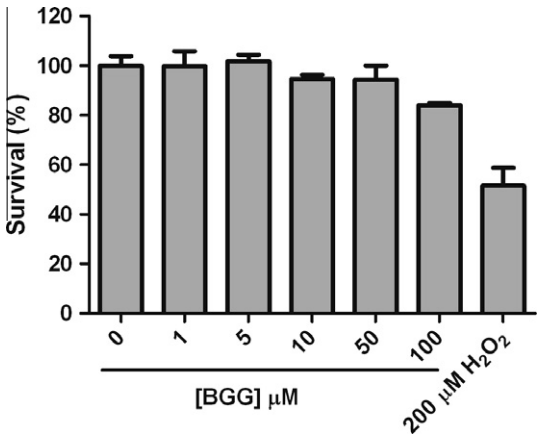

Fig. 1. The effect of BGG on the viability of Raw264.7 murine macrophages. Macrophages were treated with various concentrations of BGG for 24 h as described under Materials and Methods. As a positive control for toxicity, cells were incubated with 200 μM H_2O_2. Cell viability was determined by the Calcein Live assay. Data shown are means ± SEM ($N = 3$).