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Research article

Berberine protects against light-induced photoreceptor degeneration in the mouse retina

Delu Song [a, 1], Jiantao Song [a, b, 1], Chenguang Wang [a, c], Yafeng Li [a], Joshua L. Dunaief [a, *]

[a] The F.M. Kirby Center for Molecular Ophthalmology, Scheie Eye Institute, Perelman School of Medicine, University of Pennsylvania, United States
[b] Eye Hospital, China Academy of Chinese Medical Sciences, Beijing, China
[c] Department of Ophthalmology, The Second Hospital of Jilin University, Jilin, China

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ABSTRACT

Oxidative stress and inflammation play key roles in the light damage (LD) model of photoreceptor degeneration, as well as in age-related macular degeneration (AMD). We sought to investigate whether Berberine (BBR), an antioxidant herb extract, would protect the retina against light-induced degeneration. To accomplish this, Balb/c mice were treated with BBR or PBS via gavage for 7 days, and then were placed in constant cool white light-emitting diode (LED) light (10,000 lux) for 4 h. Retinal function and degeneration were evaluated by histology, electroretinography (ERG) and optical coherence tomography (OCT) at 7d after LD. Additionally, mRNA levels of cell-type specific, antioxidant, and inflammatory genes were compared 7d after LD. Photoreceptor DNA fragmentation was assessed via the terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay. LD resulted in substantial photoreceptor-specific cell death. Histological analysis using plastic sections showed dosing with BBR preserved photoreceptors. The ERG analysis demonstrated functional protection by BBR in rod-b, -a, and cone-b waves. In OCT images, mice receiving PBS showed severe thinning and disorganization of the photoreceptor layer 7 days after LD, whereas mice treated with BBR had significantly less thinning and disorganization. Consistent with OCT results, the mRNA levels of Rho in the NSR, and Rpe65 and Mct3 in the RPE, were significantly higher in mice treated with BBR. The numbers of TUNEL-positive photoreceptors were significantly decreased in BBR-treated mice. The retinal mRNA levels of oxidative stress genes, the number of microglia/macrophages, and the malondialdehyde (MDA) immunolabeling were significantly lower in BBR-treated mice compared to controls 48 h after LD, which indicates oxidative stress was reduced by BBR in light-damaged eyes. In conclusion, systemic BBR is protective against light-induced retinal degeneration associated with diminished oxidative stress in the retina. These results suggest that BBR may be protective against retinal diseases involving oxidative stress.

1. Introduction

In age-related macular degeneration (AMD), photoreceptor cell death may be promoted by photo-oxidative stress, and oxidative damage has been strongly implicated in AMD pathogenesis (Zarbin, 2004). Light damage (LD) in rodents has been used for over 40 years as a model of oxidative stress-induced photoreceptor degeneration, and to test antioxidants for retinal protection (Noell et al., 1966; Shahinfar et al., 1991).

Berberine (BBR) is an isoquinoline alkaloid isolated from *Coptidis rhizoma*, which is widely used in Chinese herbal medicine. BBR has a variety of biological activities including antidiarrheal, antimicrobial, antioxidant and anti-inflammatory effects (Kuo et al., 2004; Sack and Froehlich, 1982; Stermitz et al., 2000). Recent findings showed that BBR prevents neuronal damage in *in vitro* and *in vivo* models of Alzheimer's disease (Asai et al., 2007; Jiang et al., 2015; Panahi et al., 2013; Zhu and Qian, 2006). Additionally, published studies showed BBR can inhibit oxidative stress in various models, including hepatic ischemia/reperfusion injury in rats (Sheng et al., 2015), high glucose and high fat diet-induced diabetic hamsters (C. Liu et al., 2015), mercury-induced neurotoxicity in rats (Abdel Moneim, 2015), as well as endoplasmic reticulum stress in spontaneously hypertensive rats (L. Liu et al., 2015).

* Corresponding author. 305 Stellar Chance Labs, 422 Curie Blvd, Philadelphia, 19104, PA, United States.
E-mail address: jdunaief@mail.med.upenn.edu (J.L. Dunaief).
[1] These authors contributed equally to this work.

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Because oxidative stress is an important mechanism involved in light-induced photoreceptor damage, the aim of the present study was to investigate whether BBR might protect against retinal oxidative stress, inflammation and photoreceptor cell death induced by LD in wild-type Balb/c mice. We examined retinas with or without BBR treatment at day 2 and day 7 following LD. In addition, we studied the effect of BBR on mRNA levels of genes upregulated by retinal oxidative stress and inflammation.

2. Materials and methods

2.1. Animals and BBR administration

Male albino Balb/c mice, aged 10 weeks, were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were fed a standard laboratory diet, had free access to water and were maintained in a temperature-controlled room at 21–23 °C with a 12 h:12 h light–dark photoperiod. Mice underwent daily gavage with BBR (200 mg/kg/day, B3251 Sigma, St Louis, MO) in PBS or PBS alone for 1 week pre-illumination and also post-illumination until sacrifice. Experimental procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmology and vision research. All protocols were approved by the animal care review board of the University of Pennsylvania.

2.2. Light-damage paradigm

Without any prior dark adaptation, mice were exposed to 10 k lux of cool white light-emitting diode (LED) light in a well-ventilated room continuously for 4 h from 12:00 PM to 4:00 PM. LED arrays (4NFLS-x2160-24V-x, Superbrightled, St. Louis Missouri) were placed outside the cage, above and on both sides, at a distance of 25 cm from the center of the cage. The maximum irradiance was in the blue band (~380–485 nm), at 43 Watt/m^2. After the exposure to light, mice were placed in the normal light/dark cycle for 2 or 7 days. Eyes were enucleated for qPCR and terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) analysis 2 days after light exposure, and analyzed by qPCR, optical coherence tomography (OCT), electroretinography (ERG) and histology 7 days following LD.

2.3. Morphologic analysis

At day 7 following LD, enucleated eyes were immersion-fixed in 2% paraformaldehyde/2% glutaraldehyde overnight. For standard histology, 3-μm-thick plastic sections were cut in the sagittal plane and toluidine blue-stained as we have described (Song et al., 2012). The number of nuclei per column of outer nuclear layer (ONL) photoreceptors was counted in triplicate at 200 μm intervals from the optic nerve head (ONH) to 2000 μm from the ONH, using image analysis software (ImagePro Plus 4.1; Media Cybernetics) to calculate distances from manually set lengths.

2.4. Spectral domain OCT imaging

On day 7 after LD, mice were anesthetized, and their pupils were dilated with 1% tropicamide for OCT imaging. As described previously (Song et al., 2014a), artificial tears were used throughout the procedure to protect the corneas and maintain corneal clarity, and mice were seated in the Bioptigen AIM-RAS holder. Spectral domain OCT images were obtained with the Envisu R2200-HR SD-OCT device (Bioptigen, Durham, NC). Image acquisition software was provided by the vendor. One horizontal line scan 0.4 mm above the superior edge of the optic disc was saved. Corresponding ONL thicknesses for non-light damge (NLD), BBR- and PBS-treated mice eyes were compared at the same location.

2.5. Quantitative real-time PCR

To isolate the NSR and RPE cells, the eyes were washed and immersed in 2% w/v dispase in HBSS + solution at 37 °C for 40 min after enucleation. After the cornea was removed by cutting at the limbus with Vannas scissors, eyes were put in 1 mg/ml Hyaluronidase in HBSS- solution at 37 °C for 10 min to facilitate separation of the RPE from the neural retina. Next, the iris, lens, ciliary body were removed by cutting with Vannas scissors at the pars plana, and the eye cup was transferred to a 30 mm dish containing 0.5% BSA/HBSS-. The NSR was separated from the eye cup with blunt forceps, cutting at the optic nerve with Vannas scissors. It was stored at −80 °C. Holding the optic nerve with forceps, RPE cells were shaken and squeezed out of the eye cup, collected in an Eppendorf tube, and then centrifuged at 1200*g* at 4 °C for 10 min and stored at −80 °C after removal of the solution. RNA isolation was performed (RNeasy Kit; Qiagen, Valencia, CA) according to the manufacturer's protocol. cDNA was synthesized with reverse transcription reagents (TaqMan; Applied Biosystems, Darmstadt, Germany) according to the manufacturer's protocol. Gene expression in the NSR and RPE samples obtained from BBR-treated and PBS-treated mice after LD, as well as NLD mice, were analyzed by quantitative RT-PCR. Probes used were heme oxygenase 1 (Hmox1, Mm00516005_m1), ceruloplasmin (Cp, Mm00432654_m1), catalase (Cat, Mm00437858_m1), allograft inflammatory factor 1 (Aif1, Mm00479862_g1), glutathione peroxidase 1 (Gpx1, Mm00656767_g1), superoxide dismutase 1 (Sod1, Mm01344233_g1), rhodopsin (Rho, Mm01184405_m1), retinal pigment epithelium 65 (Rpe65, Mm00504133_m1) and monocarboxylic acid transporter, member 3 (Mct3, Mm00446102_m1). To quantify the expression of target genes, eukaryotic 18S rRNA (Hs99999901_s1) was used as an endogenous control. Real-time qPCR (Taqman; ABI) was performed on a sequence detection system (Prism Model 7500; ABI) using the ΔΔCT method, which provides normalized expression values. All reactions were performed on samples from four mice. For each mouse technical triplicates were analyzed. Reactions using the Rpe65 probe on neural retina and Rho probe on RPE indicate minimal cross-contamination of the isolated tissues (not shown).

2.6. Electroretinography

Electroretinography (ERG) recordings followed procedures described previously (Song et al., 2014b). In brief, mice were dark-adapted overnight and then anesthetized with a cocktail delivering (in mg/kg body weight) 25 ketamine, 10 xylazine, and 1000 urethane. Pupils were dilated with 1% tropicamide saline solution (Mydriacyl; Alcon), and mice were placed on a stage maintained at 37 °C. Two electrodes made of UV-transparent plastic with embedded platinum wires were placed in electrical contact with the corneas. A platinum wire loop placed in the mouth served as the reference and ground electrode. The ERGs were then recorded (Espion Electrophysiology System; Diagnosys LLC, Lowell, MA, USA). The apparatus was modified by the manufacturer for experiments with mice by substituting light-emitting diodes with emission maximum at 365 nm for standard blue ones. The stage was positioned in such a way that the mouse's head was located inside the stimulator (ColorDome; Diagnosys LLC), thus ensuring uniform full-field illumination. The flash intensities for recordings of rod a- and b-waves were 500 and 0.01 scot cd m^{-2} s delivered by the white xenon flash and green (510 nm maximum) LED, respectively. The white flash intensity of the cone b-wave is