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

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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 hours. 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 48h 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

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associated with diminished oxidative stress in the retina. These results suggest that BBR may be protective against retinal diseases involving oxidative stress.

Keywords

Berberine; light damage; retina; photoreceptor degeneration; oxidative stress

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).

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.

Materials and methods

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 (200mg/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.

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 4h 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 to 485nm), at 43 Watt/m². 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.

Morphologic analysis

At day 7 following LD, enucleated eyes were immersion-fixed in 2% paraformaldehyde/2% glutaraldehyde overnight. For standard histology, 3- μ m-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 μ m intervals from the optic nerve head (ONH) to 2000 μ m from the ONH, using image analysis software (ImagePro Plus 4.1; Media Cybernetics) to calculate distances from manually set lengths.

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 0.4mm above the superior edge of the optic disc was saved. Corresponding ONL thicknesses for non-light damage (NLD), BBR- and PBS-treated mice eyes were compared at the same location.

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 40min after enucleation. After the cornea was removed by cutting at the limbus with Vannas scissors, eyes were put in 1mg/ml Hyaluronidase in HBSS-solution at 37°C for 10min 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 30mm 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 1200g at 4°C for 10min 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 $\Delta\Delta C_T$ 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).

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 (Mydracil; 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 (510nm maximum) LED, respectively. The white flash intensity of the cone b-wave is 500 scot cd m⁻² s with a rod-suppressing steady green background of 30 scot cd m⁻² s.

TUNEL analysis

Following enucleation 48h following light damage, eyes were immersion-fixed in 4% paraformaldehyde (PFA) for 10 min. Cryosections were cut in the sagittal plane through the ONH. The fluorescein-conjugated TUNEL in situ cell death detection kit (Roche, Mannheim, Germany) was used for these sections, followed by fluorescence microscopy using a Nikon Eclipse TE-300 microscope (Nikon Inc., Melville, NY, USA). For each retina, the number of TUNEL-positive photoreceptors was counted in 3 sections, including a section with ONH, a section 300µm temporal to the ONH, and a section 300µm nasal to the ONH. The mean number of TUNEL-positive photoreceptors per retina was compared within BBR-treated and PBS-treated LD controls with GraphPad Prism 6.0 (San Diego, CA).

Immunofluorescence

At 48h after light damage, mice were euthanized and eyes enucleated. Eyes were then immersion fixed in 4% paraformaldehyde for 10min. Eye cups were generated by removing the anterior segment under a dissecting microscope. Eye cups (consisting of retina, RPE, choroid and sclera) were then infiltrated in 30% sucrose overnight. They were then embedded in Tissue-Tek OCT (Sakura Finetek, Torrance, CA, USA). Immunofluorescence was performed on 10- μ m-thick sections. The primary antibodies were rabbit anti-Iba1 (#019-19741, Wako, Japan) at 1: 500 dilution and rabbit anti-Malondialdehyde (MDA) (#MDA11-S, Alpha Diagnostic, San Antonio, TX) also at 1:500 dilution. Primary antibody was detected using fluorophore-labeled secondary antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Control sections were treated identically but without primary antibody. The sections were analyzed by fluorescence microscopy with identical exposure parameters (model TE300 microscope, Nikon, Tokyo, Japan) with ImagePro software (Media Cybernetics, Silver Spring, MD, USA). For quantification of the number of Iba1-positive microglia and MDA fluorescence intensity, 3 sections, including a section with ONH, a section 300 μ m temporal to the ONH, and a section 300 μ m nasal to the ONH were counted. The mean number of Iba1-positive microglia and mean fluorescence intensity of MDA immunostaining per retina was compared within BBR-treated and PBS-treated LD controls with GraphPad Prism 6.0.

Statistical analysis

The means $\pm$ SD were calculated for each comparison pair. Statistical analyses for qPCR, ONL measurement in OCT images, TUNEL-positive quantification, ERG and immunostaining intensity were performed in GraphPad Prism 6.0 (San Diego, CA) by the one-way ANOVA with a Tukey post test comparing the mean of each group with the mean of every other group. Comparison of ONL thickness (nuclei) in plastic sections was performed using one-way ANOVA with post hoc pairwise comparisons using Bonferroni adjustment. $P < 0.05$ was considered to be statistically significant.

Results

Preservation of photoreceptor nuclei by BBR

Morphologic analysis was performed 7 days following LD and the numbers of photoreceptor nuclei were quantified in sagittal sections through the ONH. BBR provided significant preservation of photoreceptors. The mice treated with BBR had more photoreceptor nuclei in the ONL and photoreceptor inner/outer segments compared to control light damaged mice treated with PBS (Fig1. A–C). As we previously described (Zhao et al., 2014), the most severely degenerated part of the retina was located on the superior side from 200 μ m to 800 μ m away from the ONH (Fig1. D).

OCT imaging at Day 7 after LD

The retinas were imaged *in vivo* by OCT. In retinas from NLD mice (Fig 2A), there are two relatively dark bands labeled INL and ONL (double-headed red arrow in Fig 2A). In retinas from control PBS-treated, light damaged mice (PBS-LD), the ONL was significantly thinner

(yellow asterisks in Fig 2B), as were the inner and outer segments (IS/OS) (green asterisks in Fig 2B). However, in BBR-treated, light damaged retinas (BBR-LD) (Fig 2C), the ONL (double-headed red arrow in Fig 2C) and IS/OS were partially protected. All horizontal line scans were taken from the superior retina 0.4mm above superior rim of the ONH (Fig 2D). Quantification of the ONL thickness is shown in Fig 2E. Compared to NLD retinas, the ONL thickness in PBS-LD control mice was significantly reduced (** $P<0.01$), whereas, BBR-LD mice had significantly thicker ONL compared to PBS-treated, light-damaged controls (** $P<0.01$).

The mRNA levels of Rho and Rpe65 at Day 7 after LD

The relative mRNA levels of Rho, Rpe65 and Mct3 were used as a measure of photoreceptor and RPE viability/differentiation. Relative to NLD retinas, PBS-LD retinas had significantly lower Rho mRNA levels (** $P<0.01$). However, BBR-LD mice had significantly higher Rho mRNA levels in the NSR (* $P<0.05$) compared to PBS-LD mice (Fig 3A). Similarly, BBR also significantly protected against the reduction of Rpe65 and Mct3 mRNA levels in RPE cells (* $P<0.05$) (Fig 3B and C). Although Rho, Rpe65 and Mct3 mRNA levels were higher in BBR-LD retinas compared to PBS-LD controls, they were still significantly lower than NLD retinas (** $P<0.01$), consistent with partial protection.

Retinal function analysis at Day 7 by ERG

To evaluate the retinal function, full-field ERG was employed. At day 7 after LD, PBS-LD mice showed significant reduction in rod-b, rod-a and cone-b waves relative to NLD mice (** $P<0.01$) (Fig 4A–F). However, BBR-LD mice had significantly higher amplitudes in rod-b, rod-a and cone-b waves compared to PBS-LD controls (* $P<0.05$). Consistent with qPCR results for Rho, Rpe65 and Mct3, all waves in BBR-treated mice had lower amplitudes compared to the NLD group (** $P<0.01$).

Numbers of TUNEL-positive photoreceptors were diminished by BBR after LD

We used the TUNEL assay to assess DNA fragmentation at day 2 after LD and quantified the TUNEL-positive photoreceptors in mice with or without BBR treatment. At day 2 after LD, many TUNEL-positive photoreceptors were observed in PBS-treated control retinas (green in Fig 5A), but only a few TUNEL-positive photoreceptors were present in retinas of BBR-LD mice (green in Fig5B). The number of TUNEL-positive photoreceptors was significantly higher in PBS-LD mice relative to NLD controls (* $P<0.05$). However, the BBR-LD mice had significantly fewer TUNEL-positive photoreceptors than PBS-LD controls (Fig5C) (* $P<0.05$).

BBR inhibited the oxidative stress and inflammation markers induced by LD

To investigate the effect of BBR on oxidative stress and inflammation gene expression in the neural retina, we performed qPCR to detect heme oxygenase 1 (Hmox1), ceruloplasmin (Cp), catalase (Cat), glutathione peroxidase 1 (Gpx1), superoxide dismutase 2 (Sod2) and allograft inflammatory factor 1 (Aif1). The mRNA levels of Hmox1, Cp, Cat, Aif1, Gpx1 and Sod1 were significantly increased in PBS-treated retinas at day 2 after LD, compared to NLD controls (** $P<0.01$). Whereas, in BBR-LD neural retinas, the mRNA levels of Hmox1,

Cp, Cat, Aif1 and Gpx1 were all significantly lower compared to PBS-treated controls (* $P<0.05$, ** $P<0.01$). In addition, compared with NLD retinas, the mRNA levels of Hmox1, Cp, Cat, Aif1 and Gpx1 were still significantly higher in BBR-LD retinas (* $P<0.05$, ** $P<0.01$) (Fig 6A–F).

BBR inhibited the microglia/macrophage infiltration induced by light damage

Following light damage, microglia/macrophage infiltration into the retina has been observed, which is consistent with previous reports (Song et al., 2012). Iba1-positive microglia/macrophages were observed in both PBS-LD (Fig 7B) and BBR-LD retinas (Fig 7C), while no staining was observed in no primary controls (not shown). Compared to NLD retinas (Fig 7A), the number of microglia/macrophages increased significantly in LD retinas (Fig 7D). However, in BBR-LD retinas, the numbers was significantly lower compared to PBS-LD retinas (Fig 7D) (* $P<0.05$, ** $P<0.01$).

BBR inhibited the LD-induced increase in MDA, an oxidative stress marker

At day 2 after light damage, immunoreactivity of MDA was increased in PBS-treated retinas (Fig 8B), compared to NLD retinas. However, it was lower in BBR-LD retinas (Fig 8C). Quantification of mean pixel density showed that light damage significantly increased MDA staining, and BBR treatment significantly inhibited this increase (Fig 8D).

Discussion

In the present study, we investigated whether systemic administration of BBR is an effective prevention for light-induced retinal degeneration. We found that BBR significantly preserved the ONL and inner/outer segments compared to PBS-treated, light damaged (PBS-LD) control mice, as measured by OCT and histology. This was further supported by a reduction of TUNEL-positive photoreceptors induced by light damage in BBR-LD mice. ERG revealed BBR protected retinal function. The LD-induced increase in oxidative stress was diminished by BBR.

Interestingly, both RPE65 and Mct3 were down-regulated in LD retinas. The reduction in RPE65 by LD agrees with our previously published data (Song et al., 2012). It indicates LD may lead to direct damage of RPE cells, which could cause down-regulation of these genes. Alternatively, the RPE may be compensating to reduce phototransduction by limiting retinal isomerization through RPE65. Similarly, RPE cells may down-regulate Mct3 to diminish photoreceptor current, which might protect against light damage (Daniele et al., 2008).

Prior studies showed that BBR inhibited increased oxidative stress in 3T3-L1 cells and increased antioxidant glutathione peroxidase gene expression and activity (Dong et al., 2015). Two independent recent studies showed BBR protects the liver from ethanol-induced oxidative stress both in mice (Zhang et al., 2014) and rats (Patil et al., 2015). The neuroprotective effect of BBR against LD may result from diminished oxidative stress and inhibition of microglial activation. Previously, we reported that two oxidative stress markers, Hmox1 and Cp, were up-regulated following photo-oxidation in the mouse retina (Chen et al., 2004, 2003). Our qPCR data showed BBR significantly inhibited the mRNA levels of Hmox1 and Cp in NSR relative to PBS-treated controls. Further, catalase and glutathione

peroxidase are both important antioxidant enzymes that were decreased in BBR-LD mice compared to PBS-LD. Additionally, increased MDA immunostaining in LD retinas was diminished in retinas from mice treated with BBR. Aif1, which encodes the protein Iba-1, is expressed microglia/macrophages and was up-regulated after LD (Zhang et al., 2005). The number of microglia in the photoreceptor layer increases markedly between 48–72hrs post-light exposure (Zhang et al., 2005). At day 2 after LD, we found both the mRNA level of Aif1 and number of Iba1-positive microglia were significantly lower in BBR-LD NSR than PBS-LD, supporting an anti-inflammatory function of BBR.

It has been shown that the PI3K (Phosphatidylinositol 3-kinase) /AKT (Serine/threonine protein kinase B) /ERK (extracellular signal-regulated kinases) pathway plays a major role in ultraviolet-induced RPE damage (Chou et al., 2013). Activation of the PI3K/AKT/ERK pathway by 17-estradiol (Mo et al., 2013) and bacterial lipopolysaccharide (Bordone et al., 2012) showed neuroprotection in the light damage model. Interestingly, published studies showed BBR can protect the hippocampus from ischemia-induced apoptosis via enhancing phospho-PI3K and phospho-Akt production (Kim et al., 2014). Similarly, damage of endothelial progenitor cells in the coronary arteries was prevented by BBR through activation of the PI3K/AKT signaling pathway (Xiao et al., 2014). It is plausible that BBR similarly protects retina from light-induced degeneration by activating the PI3K/AKT/ERK pathway.

The anti-inflammatory effect of BBR has been studied widely as well. BBR protected against retinal endothelial cell damage caused by leukocyte inflammation in diabetic retinopathy (Tian et al., 2013). BBR also appears protective against several autoimmune diseases, including rheumatoid arthritis (Hu et al., 2011), experimental autoimmune encephalomyelitis (Ma et al., 2010; Qin et al., 2010), and Vogt-Koyanagi-Harada (VKH) (Yang et al., 2013) (Cui et al., 2007). Consistent with the above findings, our qPCR data with microglia/macrophage marker, Aif1, as well as immunolabeling for microglia/macrophages, similarly indicated that BBR can reduce monocyte recruitment induced by light damage.

BBR has been used for treatment of gastrointestinal disease in China for decades, including acute gastroenteritis and bacillary dysentery. No serious safety concerns have been reported. Our findings, together with previous studies, suggest BBR, upon further testing of safety and efficacy could prove protective for eye diseases involving oxidative stress, including AMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

BBR protects photoreceptors against light damage.

BBR diminishes light-induced retinal antioxidant gene up-regulation.

BBR diminishes light-induced inflammatory gene up-regulation in the retina.

Fig 1.

Photomicrographs of plastic sections of mouse retinas and plots showing histologic protection 7 days after LD in BBR-treated retinas. Retinas from light damaged mice without BBR treatment (A), with BBR treatment (B), and non-light damaged (NLD) mice (C). White arrow heads indicate the thin ONL and red asterisks show thin inner and outer segments. Plot of the ONL thickness at day 7 after LD, measured in numbers of photoreceptor nuclei per column (D). Measurements were made in triplicate every 200 μ m from the ONH. NLD ($n = 3$, *black*), BBR-LD7 ($n = 4$, *red*), PBS control-LD7 ($n = 4$, *green*). Numbers represent mean values ($\pm$ SD). Scale bars=100 μ m (A, B, C).

Fig 2.

OCT images of mouse retinas. Compared to NLD retinas (A), light-damaged mice with PBS treatment showed striking thinning of the ONL (yellow asterisks in B) and inner/outer segments (green asterisks in B). Cross-sections of retinas in (A, B, C) were from a line scan in the superior retina 0.4mm above ONH (red circle in D). In BBR-treated retinas, both ONL (double-headed red arrow in C) and inner/outer segments were well preserved.

Quantification of ONL thickness (E) showed that PBS-treated, light damaged control retinas had significantly thinner ONL than NLD mice, while BBR protected against the thinning caused by LD. N=3 in NLD group, N=4 in both PBS-treated and BBR-treated group.

** $P < 0.01$

Fig 3.

Graphs showing relative mRNA levels by qPCR. The mRNA levels of Rho, Rpe65 and Mct3 were decreased significantly after LD. The BBR treatment resulted in a smaller reduction of Rho mRNA levels in the neural retina, as well as Rpe65 and Mct3 mRNA levels in the RPE, compared to PBS-treated controls. Numbers represent mean values ($\pm$ SD). N=3 in NLD group, N=4 in both PBS-treated and BBR-treated group. * P <0.05, ** P <0.01

Fig 4.

Full-field ERG responses of PBS-treated control and BBR-treated mice 7 days after LD. Compared to NLD mice, the PBS-treated mice had significant reduction in all waves of the ERG. Maximum amplitudes of rod-b (A), rod-a (B) and cone-b (C) waves were significantly higher in BBR-treated mice, compared to light damaged, PBS-treated controls. Numbers represent mean values ($\pm$ SD). N=3 in NLD group, N=4 in both PBS-treated and BBR-treated group. * $P < 0.05$, ** $P < 0.01$. The original traces of ERG recordings from one mouse in each group were plotted in D (rod-b), E (rod-a) and F (cone-b).

Fig 5.

Fluorescence photomicrographs showing TUNEL-positive photoreceptor in retinas. There were significantly more TUNEL-positive photoreceptors (green dots in A) at day 2 after LD in PBS-treated retinas, while BBR-treated retinas showed only a few TUNEL-positive photoreceptors (green dots in B). Bar graph showing significantly increased numbers of TUNEL-positive photoreceptors in PBS-treated retinas post-LD. The numbers of TUNEL-positive photoreceptors were significantly diminished in BBR-treated retinas compared to PBS-treated controls (C). GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retina pigmented epithelium. Scale bars in (A and B) represents 100 μ m. N=3 in NLD group, N=4 in both PBS-treated and BBR-treated group. * P <0.05

Fig 6.

Graphs showing relative mRNA levels measured by qPCR. Compared to NLD retinas, the mRNA levels of Hmox1, Cp, Cat, Aif1, Gpx1 and Sod1 were all significantly increased in PBS-treated retinas after LD. In BBR-treated retinas, the oxidative stress genes, Hmox1, Cp, Cat and Gpx1, as well as inflammation gene, Aif1 were all decreased compared to light damaged, PBS-treated controls. However, compared with NLD retinas, the mRNA levels of Hmox1, Cp, Cat, Aif1 and Gpx1 were still significantly higher in BBR-treated retinas. PBS-treated ($n = 4$) and BBR-treated ($n = 4$) retinas are displayed as mean values ($\pm$ SD).

* $P < 0.05$, ** $P < 0.01$

Fig 7.

The number of Iba1-positive microglia/macrophages was decreased by BBR after LD. Fluorescence photomicrographs and counting of Iba1-positive retinal microglia/macrophages from retinas 48h after LD. Iba1-positive retinal microglia/macrophages were found in both PBS-(B) and BBR-treated retinas (C), but only a few in control retinas without LD (A). Compared to NLD, the number of microglia/macrophages in the retina increased significantly 48h after light exposure, which is significantly diminished by BBR (D). Scale bars in (A, B and C) represents 100 μ m. NLD ($n=3$), Ctrl-LD2 ($n=3$), and BBR-LD2 ($n=4$) retinas are displayed as mean values ($\pm$ SD). * $P<0.05$, ** $P<0.01$

Fig 8.

Retinal MDA immunolabeling and mean fluorescence intensity quantification. Fluorescence photomicrographs of retinas after LD (B) showed higher immunolabeling of oxidative stress marker MDA than NLD retinas (A), however, it was diminished by BBR treatment (C). Immunostaining was quantified by measuring the mean pixel intensity within the whole retina (D). Scale bars=100 μ m. NLD ($n=3$), Ctrl-LD2 ($n=3$), and BBR-LD2 ($n=3$) retinas are displayed as mean values ($\pm$ SD). * $P<0.05$, ** $P<0.01$