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Email: paul.park@lumc.edu

Best daytime phone: *(Clinic phone, Cell phone, Pager)* 8478005980

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Paeoniflorin Protects Against Retinal Photoreceptor Degeneration in a Mouse Model of Light-induced Retinal Injury

Paul J Park[1], Crystal Algenio[2], Zhiqun Tan[3], James F McDonnell[1], Liang Qiao[4], Jay I Perlman[1,5], Ping Bu[5]

[1] Department of Ophthalmology, Loyola University Chicago Health Sciences Division, Maywood, IL 60153, USA

[2] Loyola University Stritch School of Medicine, Maywood, IL 60153, USA

[3] Institute for Neurological Impairments and Neurological Disorders, University of California, Irvine, Irvine, CA 92697, USA

[4] Department of Microbiology and Immunology, Loyola University Chicago Health Sciences Division, Maywood, IL 60153, USA

[5] Research Service, Edward Hines Jr., VA Hospital, Hines, IL 60141, USA

Abstract

Purpose:

Age-related macular degeneration (AMD) is a leading cause of blindness in the United States and other developed nations. Current treatment modalities targeting AMD are beneficial to only a small proportion of patients with the end-stage form and do not necessarily prevent progression of the disease. Paeoniflorin (PF) is one of the main active components of Paeonia Radix, a traditional Chinese herbal medicine derived from the root of Paeonia lactiflora Pallas. Paeoniflorin possesses both anti-inflammatory and anti-oxidant properties and has been implicated as a potential therapy in other degenerative pathologies such as Alzheimer's disease. We hypothesize that paeoniflorin will have protective effects against retinal degeneration in a mouse model of retinal light injury.

Methods:

BALB/c albino mice were assigned to one of two groups: control-treated or paeoniflorin-treated retinal light injury. Control (PBS) or paeoniflorin (20 mg/kg in PBS) was injected intraperitoneally and mice were then exposed to 10,000 lux cool white fluorescent light for 2 hours to induce light injury. Both groups had treatment administered once daily for 4 additional days following light injury for a total of 5 treatments. Scotopic electroretinography (ERG) was recorded before light injury and 7 days following light injury. After ERG recording 7 days following light injury, mice eyes were then enucleated and fixed in phosphate-buffered (pH 7.4) solution of 2.5% glutaraldehyde-2% paraformaldehyde. The eye cups were dissected and then embedded in epoxy resin for retinal histology and morphometry.

Results:

Before treatment, scotopic ERG a- and b-wave amplitudes were an average of 542 ± 31 μV and 1005 ± 61 μV, respectively, in the control group and 452 ± 109 μV and 867 ± 147 μV, respectively, in the paeoniflorin treatment group. Following retinal light injury, ERG a-wave amplitudes were 132 ± 17 μV in the control group and 250 ± 59 μV in the paeoniflorin treatment group ($p < 0.005$). Following retinal light injury, ERG b-wave amplitudes were 304 ± 42 μV in the control group and 518 ± 138 μV in the paeoniflorin treatment group ($p < 0.005$). Baseline a- and b-wave amplitudes between control and treated groups were statistically indistinguishable ($p > 0.05$). In control treated mice, light injury produced significant thinning of the outer nuclear layer (ONL), but the degree of thinning of the ONL was significantly less in cases of paeoniflorin treatment. There was significantly greater preservation of ERG a- and b-wave amplitudes in the paeoniflorin treatment group as well as greater histologic preservation of retinal layers.

Conclusion:

Paeoniflorin treatment of mice had a significant impact on the preservation of ERG a-and b-wave amplitudes following retinal light injury when compared to a control group. Greater histologic preservation was also seen with paeoniflorin treatment. Our preliminary findings suggest that paeoniflorin may have therapeutic value in the management of retinal degenerative conditions.

Introduction

AMD is a leading cause of blindness in the developed world. In the United States alone, an estimated 10 million Americans are affected by AMD. Current treatment modalities targeting AMD are beneficial to only a small proportion of patients with end-stage disease. Furthermore, FDA-approved treatments only exist for exudative "wet, neovascular" AMD, not for the non-exudative "dry, atrophic" form.[1] An incomplete understanding of its pathogenesis has limited progress in the development of novel therapeutics against this disease. Although mainstay treatment options include high-dose antioxidant vitamins, both the AREDS and AREDS2 formulations mainly have been shown to reduce the incidence of neovascular AMD in select individuals. No convincing data exist to suggest either of these antioxidant formulations prevent the progression to chronic geographic atrophy, the severe end-stage form of dry AMD.[2]

Paeoniflorin is one of the main active components of Paeonia Radix, a traditional Chinese herbal medicine derived from the root of *Paeonia lactiflora* Pall. Previous studies have demonstrated a protective role of paeoniflorin in a cell culture model of AMD.[3] Paeoniflorin possesses both anti-inflammatory and anti-oxidant properties and has also been implicated as a potential therapy in other degenerative pathologies such as Alzheimer's disease.[3, 4, 5] However, no studies to date have examined the therapeutic potential of paeoniflorin in a live animal model of retinal degeneration. Furthermore, the potential protective effect of this compound specifically on retinal photoreceptors, a key cellular structure that is affected in AMD, has not been investigated.

In this study we employ a live mouse model of retinal light injury to investigate the potential protective benefits of paeoniflorin against this specific type of retinal degeneration. The mouse retinal light-induced injury model has been successfully used in retinal neurodegenerative disease studies.[6] Oxidative stress, in particular, has been shown to be an important mechanism involved in light-induced retinal damage.[7] Although mice anatomically lack a macula, other components such as the neuroretina and the retinal pigment epithelium can develop lesions that portray clinical features of AMD and have been widely used in studies of potential therapeutics against this vision-threatening condition. Shibagaki, K et al. recently employed a mouse retinal light-induced injury model to investigate the potential benefits of another compound against oxidative stress in such diseases such as AMD. In that study, the compound of interest, pramipexole, was found to have a protective benefit in the animal model.[8] Studies have also confirmed that exposure of the retina to increased levels of oxidative stress in animal models leads to loss of photoreceptors and retinal pigment epithelium, two main hallmarks of AMD.[9] Given the previously

described anti-inflammatory and anti-oxidant properties of paeoniflorin, we suggest paeoniflorin may have therapeutic value in the management of retinal degenerative conditions.

Materials and Methods

IACUC Statement

All procedures involving mice were performed according to the ARVO statement for the use of Animal in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee of Edward Hines, Jr. VA Hospital.

Light-induced retinal injury

All exposure to light began at 10 AM. The BALB/c albino mice (8 weeks) were dark adapted for 24 hours before the experiments, and the pupils were dilated with 1% cyclopentolate hydrochloride eye drops 1 hour before exposure to light and right before exposure to the light. Non-anesthetized mice were exposed to 10,000 lux of diffuse, cool, white fluorescent light for 2 hours (from 10AM-12AM). A special device was used with round light bulbs to prevent the mice hiding their face. The temperature during exposure to light was maintained at 25 + 1.5°C.

Paeoniflorin Treatment

Paeoniflorin was dissolved in PBS (stock 200 mg/ml). Paeoniflorin (20 mg/kg/day) or control (PBS) was administered one time before light injury and for 4 additional days following retinal light injury via intraperitoneal injection.

Electroretinography (ERG)

Retinal function (ERG) were measured before and 7 days after inducing retinal light injury. Mice were dark-adapted overnight, anesthetized with ketamine (100mg/kg) and xylazine (5mg/kg) and their pupils dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride. Using a stainless-steel electrode coated with 1% methylcellulose, the ERG was recorded from the corneal surface with a series of stimulus luminance. Needle electrodes were subcutaneously inserted in the cheek and the tail served as reference and ground leads, respectively. The ERG responses were differentially amplified (0.3–1,500 Hz), averaged and stored using a UTAS E-3000 signal averaging system (LKC Technologies, Gaithersburg, MD). A notch filter at 60 Hz was used during recording. Stimuli ranged from -3.6 to 2.1 log cd s/m^2 and were presented

in increasing order in dark and at least two successive responses were averaged together for each stimulus presented. Intervals between the stimuli were increased from 4 s to 61 s. The body temperature of anesthetized mice was kept at 37° C using a temperature-regulated heating pad.

Histology and Morphometry

The eyes from all groups were collected after ERG recording at 7 days after inducing retinal light injury. Enucleated eyes were fixed in phosphate-buffered (pH 7.4) solution of 2.5% glutaraldehyde-2% paraformaldehyde. The eye cups were dissected and then embedded in epoxy resin. Sections with 1 µm thickness were cut vertically through the optic nerve and subsequently stained with toluidine blue. Three separate morphometric measurements of the outer nerve layer (ONL) from the superior and inferior retina between 750-1000 µm from the optic disc were averaged together. The histology changes were evaluated by measuring the outer retinal thickness in a protocol previously described.[10]

Statistical Analysis

Statistical significance between the means of multiple experimental groups will be determined using one-way analysis of variance (ANOVA). An F ratio giving a value of $p < 0.05$ is considered significant.

Results

Before treatment, scotopic ERG a- and b-wave amplitudes were an average of 542 ± 31µV and 1005 ± 61 µV, respectively, in the control group and 452 ± 109 µV and 867 ± 147 µV, respectively, in the paeoniflorin treatment group (Figure 1 and 2). Following retinal light injury, ERG a-wave amplitudes were 132 ± 17 µV in the control group and 250 ± 59 µV in the paeoniflorin treatment group ($p < 0.005$). Following retinal light injury, ERG b-wave amplitudes were 304 ± 42 µV in the control group and 518 ± 138 µV in the paeoniflorin treatment group ($p < 0.005$). There was significantly greater preservation of ERG a- and b-wave amplitudes in the paeoniflorin treatment group suggesting that paeoniflorin is able to attenuate the degree of degenerative loss of retinal function in a retinal light injury model.

Light injury induced photoreceptor degeneration and photoreceptor cell death was evidenced in toluidine blue stained sections after 7 days post retinal light injury. However, the thinning of the ONL in the paeoniflorin-treated light injury group was relatively preserved as compared to that seen in the control-treated light injury group (Figure 3). The difference in mean thickness of the ONL at 750 - 1000 µm from the optic disc between the control and the paeoniflorin-treated light injury groups was statistically significant (Figure 4).

Discussion

Our findings demonstrate that paeoniflorin treatment can protect against retinal degeneration in a mouse model of retinal light injury. This preventive effect was observed in both functional and structural studies. The exact mechanism by which paeoniflorin exerts its potential protective effect is unclear. However, as previously described, paeoniflorin possess both anti-inflammatory and anti-oxidative properties.[3, 4, 5] Given that prior studies have also confirmed that exposure of the retina to increased levels of oxidative stress in animal models leads to loss of photoreceptors and retinal pigment epithelium, which are two main hallmarks of AMD, it is not surprising that a compound with these properties is able to produce findings suggestive of a protective effect against this type of degeneration. While paeoniflorin has been demonstrated to have protective effects in other neurodegenerative conditions[3, 4, 5], we report for the first time a similar effect of paeoniflorin on retinal function. Further research to elucidate the exact mechanisms involved are warranted.

Acknowledgments

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Figures

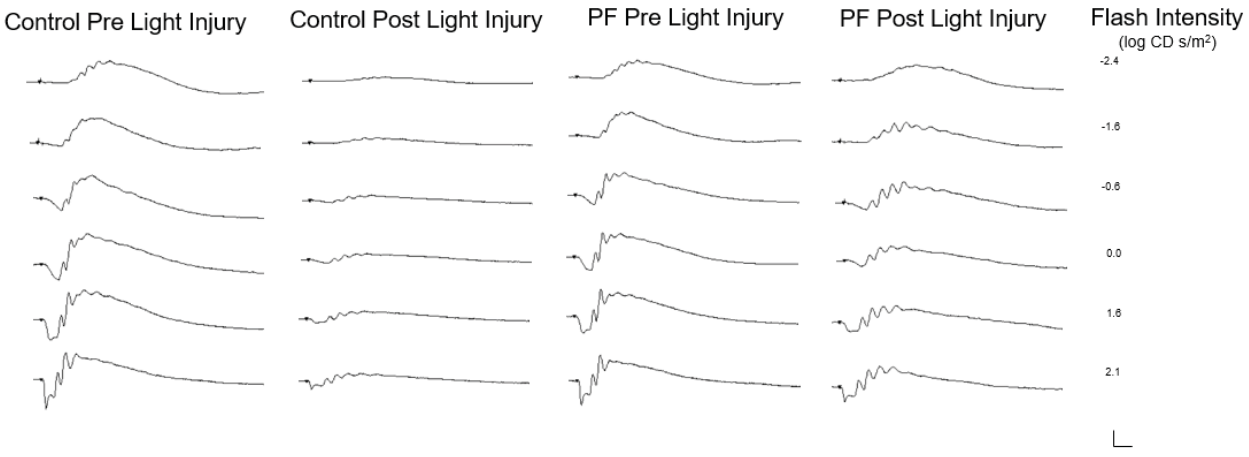

Figure 1. Representative dark-adapted ERG a- and b-waves. Preservation of a- and b-waves in the paeoniflorin treatment group compared to that of the control group is demonstrated. Scale bars: 20 ms (x-axis) and 250 μV (y-axis).

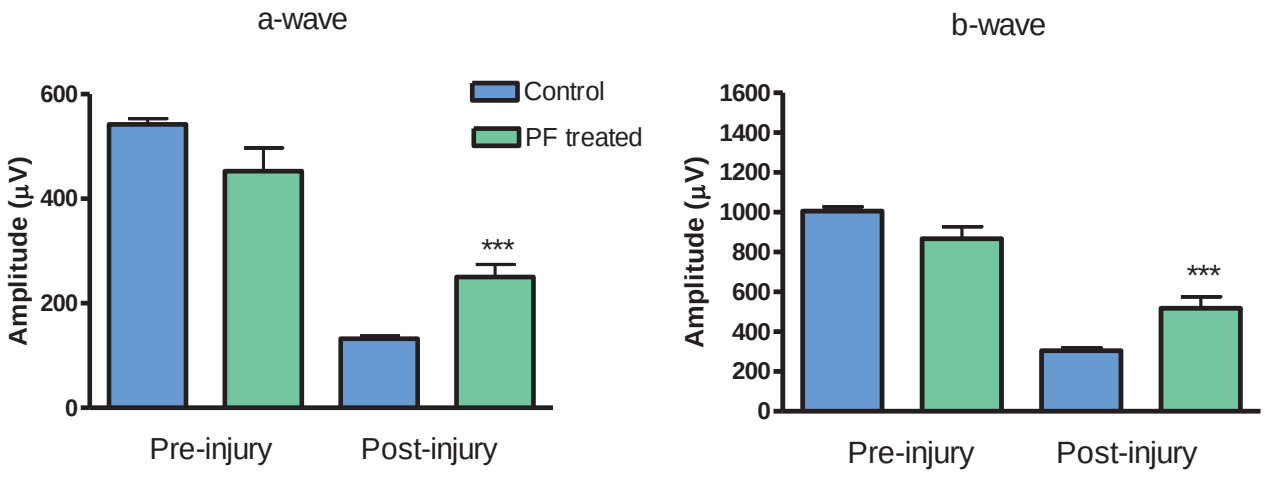

Figure 2. Effect of paeoniflorin treatment on retinal function following light injury. Quantitative changes in ERG a- and b-wave amplitudes at 2.1 log CD s/m^2 flash intensity.

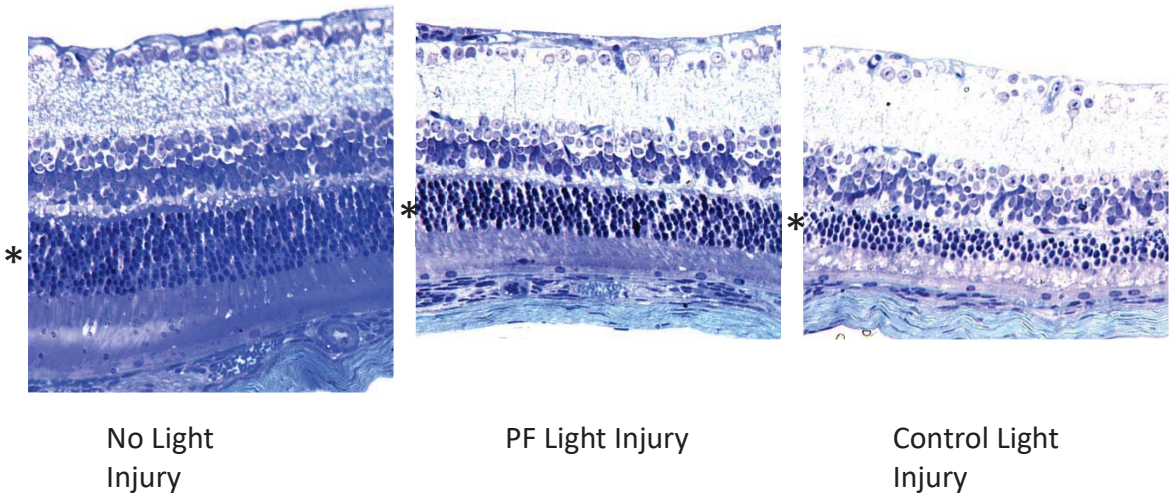

Figure 3. Effect of paeoniflorin on retinal histology following retinal light injury. Representative stained retinal sections from normal (No Light Injury), paeoniflorin-treated (PF Light Injury) or control-treated (Control Light Injury) tissues. Data shown are representative samples (n = 3). An * is used to indicate the outer nuclear layer.

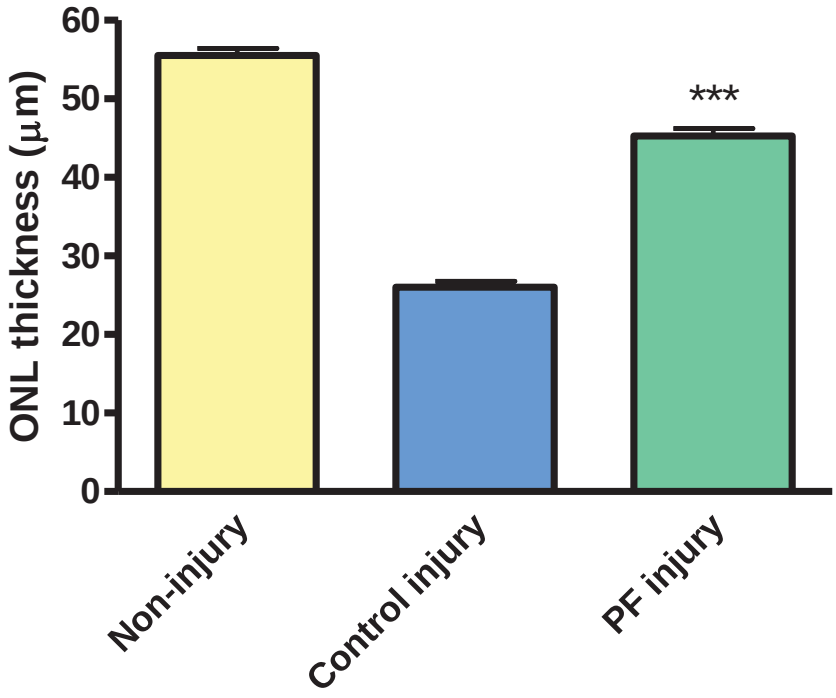

Figure 4. Morphometry data quantifying the preservation of the outer nuclear layer thickness between the no light injury (non-injury), control-treated light injury (control injury) and the paeoniflorin-treated light injury (PF injury) groups.