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Original article

Absence of DJ-1 causes age-related retinal abnormalities in association with increased oxidative stress

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ABSTRACT

Oxidative stress alters physiological function in most biological tissues and can lead to cell death. In the retina, oxidative stress initiates a cascade of events leading to focal loss of RPE and photoreceptors, which is thought to be a major contributing factor to geographic atrophy. Despite these implications, the molecular regulation of RPE oxidative stress under normal and pathological conditions remains largely unknown. A better understanding of the mechanisms involved in regulating RPE and photoreceptors oxidative stress response is greatly needed. To this end we evaluated photoreceptor and RPE changes in mice deficient in DJ-1, a protein that is thought to be important in protecting cells from oxidative stress.

Young (3 months) and aged (18 months) DJ-1 knockout (DJ-1 KO) and age-matched wild-type mice were examined. In both group of aged mice, scanning laser ophthalmoscopy (SLO) showed the presence of a few autofluorescent foci. The 18 month-old DJ-1 KO retinas were also characterized by a noticeable increase in RPE fluorescence to wild-type. Optical coherence tomography (OCT) imaging demonstrated that all retinal layers were present in the eyes of both DJ-1 KO groups. ERG comparisons showed that older DJ-1 KO mice had reduced sensitivity under dark- and light-adapted conditions compared to age-matched control. Histologically, the RPE contained prominent vacuoles in young DJ-1 KO group with the appearance of enlarged irregularly shaped RPE cells in the older group. These were also evident in OCT and in whole mount RPE/choroid preparations labeled with phalloidin. Photoreceptors in the older DJ-1 KO mice displayed decreased immunoreactivity to rhodopsin and localized reduction in cone markers compared to the wild-type control group. Lower levels of activated Nrf2 were evident in retina/RPE lysates in both young and old DJ-1 KO mouse groups compared to wild-type control levels. Conversely, higher levels of protein carbonyl derivatives and iNOS immunoreactivity were detected in retina/RPE lysates from both young and old DJ-1 KO mice.

These results demonstrate that DJ-1 KO mice display progressive signs of retinal/RPE degeneration in association with higher levels of oxidative stress markers. Collectively this analysis indicates that DJ-1 plays an important role in protecting photoreceptors and RPE from oxidative damage during aging.

1. Introduction

DJ-1 is a multifunctional protein that plays an important role in the oxidative stress response in sporadic Parkinson's disease (PD) [1–3] and in other neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) [4], Alzheimer's disease (AD) [2] and Huntington's disease (HD) [5]. Deletion of or mutations in the DJ-1 gene (*PARK7*) result in early-onset familial PD [6–8].

DJ-1 is ubiquitously expressed in many tissues, including the brain, where it functions as an antioxidant, redox-sensitive molecular chaperone and transcriptional regulator that protects cells from oxidative stress. DJ-1 has been intensely studied in neurons; however, its role in the retina and retinal pigment epithelium (RPE) cells is poorly understood. Our initial experiments identified DJ-1 peptides in RPE fractions of both young and old rats [9]. Further analysis detected higher levels of DJ-1 in RPE lysates from older animals [9]. Consistent with its role as

Abbreviations: PD, Parkinson's disease; DJ, 1 KO- DJ-1 knockout; RPE, retinal pigment epithelium; ROS, reactive oxygen species; AMD, age-related macular degeneration

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an antioxidant, we previously demonstrated that increased DJ-1 expression in RPE cultures prior to exposure to oxidative stress significantly decreased ROS generation [10]. We also reported mild structural and physiological changes in the retinas of the young vs middle-aged DJ-1 knockout (DJ-1 KO) mice in association with increased oxidative stress. These included an increase in the amplitude of the scotopic ERG b-wave (reflecting bipolar cell function), an increase in amplitude of the cone ERG, decreased amplitude of a subset of the dc-ERG components (reflecting RPE function) at 6 months of age in the KO compared to the control, RPE thinning, decreased tyrosine hydroxylase in dopaminergic neurons, and increased 7,8- dihydro-8-oxoguanine-labeled DNA oxidation [11].

Here, we report that the severity of these changes increased with age through the analysis of older (18 month-old) mice and compared the results to young control mice. We identified retinal thinning, morphological and physiological changes in the RPE and photoreceptors, and accumulation of markers of oxidative stress. Our results suggest that the DJ-1 KO mice are a model of mild chronic RPE oxidative stress that results in a progressive phenotype of age-related retinal degeneration.

2. Materials and methods

2.1. DJ-1 knockout (KO) model

Mice with a deletion of the second exon of DJ-1 have been characterized previously [12]. Mice were housed in a 12-h light/dark cycle and fed a regular diet ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC protocol number ARC 2016-1048) at the Cleveland Clinic. Homozygous DJ-1 KO mice were backcrossed onto the C57Bl/6J background to generate wild-type control mice. Mice negative for the presence of the DJ-1 PCR product were used as control. DJ-1 heterozygous KO mice were not analyzed. Analyses were carried out in both male and female 3- and 18-month-old mice. The number of mice/genotype/age examined in this study is summarized in Table 1. For in vivo imaging and retinal function experiments, mice were anesthetized with sodium pentobarbital (Nembutal sodium solution, USP, 65 mg/kg, Oak Pharmaceuticals, Inc., subsidiary of Akorn, Inc., Lake Forest, IL), and all efforts were made to minimize procedural stress.

Table 1
Experimental study groups.

Experiment	Number of Mice	Genotype	Age
In vivo imaging	5 (M)	Control	3 month-old
In vivo imaging	4 (M), 2 (F)	Control	18 month-old
In vivo imaging	2 (M), 3 (F)	DJ-1 KO	3 month-old
In vivo imaging	5 (M)	DJ-1 KO	18 month-old
Retinal function	2 (M), 1 (F)	Control	3 month-old
Retinal function	2 (M), 2 (F)	Control	18 month-old
Retinal function	3 (M), 5 (F)	DJ-1 KO	3 month-old
Retinal function	3 (M), 5 (F)	DJ-1 KO	18 month-old
Histology	4 (M), 5 (F)	Control	3 month-old
Histology	3 (M), 6 (F)	Control	18 month-old
Histology	6 (M), 5 (F)	DJ-1 KO	3 month-old
Histology	5 (M), 2 (F)	DJ-1 KO	18 month-old
RPE and retina whole-mounts	8 (M), 4 (F)	Control	3 month-old
RPE and retina whole-mounts	4 (M), 8 (F)	Control	18 month-old
RPE and retina whole-mounts	6 (M), 3 (F)	DJ-1 KO	3 month-old
RPE and retina whole-mounts	7 (M), 3 (F)	DJ-1 KO	18 month-old
Elisa / Western blots	2 (M), 3 (F)	Control	3 month-old
Elisa / Western blots	2 (M), 2 (F)	Control	18 month-old
Elisa / Western blots	2 (M), 3 (F)	DJ-1 KO	3 month-old
Elisa / Western blots	1 (M), 3 (F)	DJ-1 KO	18 month-old

M- male, F- female

2.2. Genotyping

Genomic DNA was isolated from tail biopsies using DirectPCR Lysis Reagent (mouse tail, Viagen Biotech, Los Angeles, CA) and subjected to PCR amplification using specific sets of PCR primers for the DJ-1 genotype as previously reported [11]. We confirmed the absence of Crb1 rd8 mutation in our mice by PCR amplification as previously described [13].

2.3. In vivo imaging of retinas

Imaging by confocal scanning laser ophthalmoscopy (model HRA2; Heidelberg Engineering, Inc., Vista, CA) and spectral-domain optical coherence tomography (SD-OCT) (model SDOIS; Bioptigen, Inc., Research Triangle Park, NC) was carried out following sodium pentobarbitol anesthesia and pupil dilation with 1.5 μL of 0.5% Mydrin-P drops (Santen Pharmaceutical Co., Ltd., Osaka, Japan) and topical anesthesia 0.5% proparacaine HCl as previously described [15]. Autofluorescence SLO (AF-SLO) images were obtained using an excitation wavelength of 488 nm. SD-OCT images from the horizontal and vertical meridian were collected using 1000 A-scans/B-scan x 10 B-scans. B-scans from each meridian were co-registered and averaged using ImageJ. In-depth profiles (see Supplement Fig. S1) of retinal morphology, extracted from SD-OCT B-scans were analyzed as previously described [11].

2.4. Retinal function

Retinal function of young and aged DJ-1 KO and control mice was studied after overnight dark adaptation as previously described [11]. Mice were anesthetized as described above. Eye drops were used to anesthetize the cornea (1% proparacaine HCl) and to dilate the pupil (2.5% phenylephrine HCl, 1% tropicamide, and 1% cyclopentolate HCl). Mice were placed on a temperature-regulated heating pad throughout the recording session. Electroretinogram (ERG) components generated by the neural retina were measured in response to strobe flash stimulation using a recording protocol that has been developed for mice as previously described [16]. Strobe flash ERGs were recorded using a stainless steel electrode in contact with the corneal surface with 1% methylcellulose. Needle electrodes were placed in the cheek for the reference and in the tail for the ground leads. Dark-adapted responses were presented within an LKC ganzfeld and recorded using increasing flash intensities from 3.6 to 2.1 log cd s/m^2. The number of successive responses averaged together decreased from 20 for low-intensity flashes to 2 for the highest intensity stimuli. Conversely, the duration of the interstimulus interval (ISI) increased from 4 s for low-intensity flashes to 90 s for the highest-intensity stimuli. Responses were differentially amplified (0.3–1500 Hz), averaged, and stored using a UTAS E-3000. The direct-current coupled (dc)–ERG components generated by the RPE in response to a 7 min duration stimulus were also recorded and analyzed as previously described [17]. Briefly, white light stimuli were derived from an optical channel using a Leica microscope illuminator as the light source and delivered to the test eye with a 1-cm-diam fiber optic bundle. Thirty seconds of baseline recording prior to the stimulus and 90 s following the light off were recorded to capture the c-wave, fast oscillation, light peak and off responses.

2.5. Histology and transmission electron microscopy of retinas

Eyes were enucleated and fixed by immersion in 2% paraformaldehyde, 2.5% glutaraldehyde and 5% $CaCl_2$ made in 0.1 M cacodylate buffer overnight at 4 °C and processed for epon embedding as previously described [11]. For bright-field microscopy, semi-thin sections were cut with a diamond histotech knife, collected on glass slides, and stained with toluidine blue. Slides were photographed with a Zeiss AxioImager. Z1 light microscope and AxioCam MRc5 camera. For