Nutrition 32 (2016) 384–390

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Protective effects of a grape-supplemented diet in a mouse model of retinal degeneration

Amit K. Patel Ph.D., Ashley Davis B.Sc., Maria Esperanza Rodriguez M.D., Samantha Agron, Abigail S. Hackam Ph.D. *

Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, USA

ARTICLE INFO

Article history:
Received 18 May 2015
Accepted 17 September 2015

Keywords:
Retinal degeneration
Freeze-dried grape powder
Oxidative stress
Paraquat
Neuroprotection
Nutrition
Retina
Grapes

ABSTRACT

Objective: Retinal degenerations are a class of devastating blinding diseases that are characterized by photoreceptor dysfunction and death. In this study, we tested whether grape consumption, in the form of freeze-dried grape powder (FDGP), improves photoreceptor survival in a mouse model of retinal degeneration.

Methods: Retinal degeneration was induced in mice by acute oxidative stress using subretinal injection of paraquat. The grape-supplemented diet was made by formulating base mouse chow with FDGP, corresponding to three daily human servings of grapes, and a control diet was formulated with equivalent sugar composition as FDGP (0.68% glucose–0.68% fructose mixture). Mice were placed on the diets at weaning for 5 wk before oxidative stress injury until analysis at 2 wk post-injection. Retinal function was measured using electroretinography, thickness of the photoreceptor layer was measured using optical coherence tomography, and rows of photoreceptor nuclei were counted on histologic sections.

Results: In mice fed the control diet, oxidative stress significantly reduced photoreceptor layer thickness and photoreceptor numbers. In contrast, retinal thickness and photoreceptor numbers were not reduced by oxidative stress in mice on the grape-supplemented diet, indicating significantly higher photoreceptor survival after injury than mice on the control diet. Furthermore, mice on the grape diet showed preservation of retinal function after oxidative stress injury compared with mice on the control diet.

Conclusions: A diet supplemented with grapes rescued retinal structure and function in an oxidative stress–induced mouse model of retinal degeneration, which demonstrates the beneficial effect of grapes on photoreceptors.

Introduction

Retinal degenerations are a class of diseases that cause progressive photoreceptor death and irreversibly impair vision. These diseases include age-related macular degeneration (AMD) and retinitis pigmentosa (RP), and have high prevalence throughout the world. In the United States, AMD affects over 1.75 million people [1] and RP affects every 1 in 4000 individuals [2]. A major focus in the field is identifying new therapeutic strategies that promote photoreceptor survival. Nutrition- and diet-based therapies are of particular interest because they have the potential to ameliorate progression of the disease with potentially fewer side effects than pharmacotherapy.

Numerous studies have explored the benefits to the retina of specific dietary supplements, ingested either as isolated compounds or as whole foods. A large randomized controlled trial found that, in patients with AMD, progression to advanced disease was delayed by ingesting certain nutrients, including antioxidant vitamins and specific carotenoids [3]. This finding led to the development of the Age-Related Eye Disease Study (AREDS) dietary supplement formulation that is recommended to patients with AMD and is widely available commercially, which includes a combination of vitamins C and E, zinc, and beta-carotene or lutein and zeaxanthin. Various other nutritional supplements, such as minerals, dietary fatty acids, and resveratrol, have also been associated with improved retinal health in

* Corresponding author. Tel.: +1 305 547 3723; fax: +1 305 547 3658.
E-mail address: ahackam@med.miami.edu (A. S. Hackam).

http://dx.doi.org/10.1016/j.nut.2015.09.017
0899-9007/

small-sample observational studies, although those tested in subsequent larger studies often show lack of statistically significant benefits [4]. However, an overall healthy diet, which includes low fat and high fruit and vegetable intake, appears to be important for reducing progression of retinal degeneration [5].

Grapes are an ideal candidate for dietary supplementation because polyphenols and other molecules isolated from grapes have anti-oxidant and anti-inflammatory effects in numerous tissues [6]. Oxidative damage and inflammation contribute to retinal degenerations [7,8], leading to the hypothesis that the bioactive components found in grapes could protect photoreceptors. Grape consumption and grape-derived compounds are reported to be neuroprotective elsewhere in the central nervous system [9], and in mice with a retinal pigment epithelium (RPE)–specific phagocytosis defect, whole grapes prevented RPE damage and secondary retinal dysfunction [10], indicating that grape-derived compounds are biologically active in the retina. However, the effect of grape consumption on the neural retina, especially on photoreceptors during retinal degeneration has not previously been examined.

Characterizing basic mechanisms of photoreceptor survival in simple models of retinal degeneration in animals is frequently used to identify novel therapeutic strategies that could be relevant for complex degenerations in humans such as AMD. Elevated oxidative stress is strongly associated with retinal disease and has been widely studied in the development of AMD and other retinal degenerations. An acute oxidative stress–induced retinal degeneration model is commonly used to mimic oxidative stress damage that contributes to retinal degenerations [11]. Oxidative stress injury is induced by subretinal injection of paraquat (PQ) in mice and has the advantage as a non-genetic model that it does not require complex breeding schemes. PQ is processed in the mitochondria into free radicals [12] and induces oxidative damage when injected directly into the retina [11]. We previously reported that PQ induced retinal degeneration in mice, and reduced retinal thickness, lowered photoreceptor counts, attenuated electroretinography (ERG) responses, and decreased visual acuity, which could all be prevented by delivery of specific molecules [13].

In this study, we characterized the effect of dietary supplementation of grapes in a mouse model of oxidative stress–induced retinal degeneration. We demonstrated that mice fed a grape-enriched diet, in the form of freeze-dried grape powder (FDGP), showed remarkably improved photoreceptor survival at both the structural and functional levels. Therefore, our data provide new evidence for a beneficial effect of grapes on photoreceptor health. These results suggest that dietary grape intake may be a viable preventative or adjuvant therapy for retinal degenerations.

Materials and methods

Diets

All procedures involving mice were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care and Use Committee at the University of Miami. Dietary supplementation with grapes was achieved using a biochemically characterized FDGP that was prepared from California whole grapes and seeds of different varieties—red, green, and black—and was provided by the California Table Grape Commission. FDGP has been used in numerous studies for testing the effect of grapes in human and animal studies [6,10,14]. The biochemical composition of FDGP has previously been described [15,16]. FDGP is composed of fresh red, green, and black California grapes (seeded and seedless varieties) that have been frozen, ground with food-quality dry ice, freeze-dried, and reground using Good Manufacturing Practices for food products. The FDGP was incorporated into AIN-76A base diet mouse chow (prepared by Purina Mills, Gray Summit, MO, USA) to provide approximately 43 mg of FDGP per day, based on the reported average amount of food ingested per mouse per day, and corresponds to approximately three human servings of grapes (USDA serving size is one cup of grapes per serving) [10]. This dose is similar to what was used in a recent report to allow direct comparisons [10]. A control diet that was sugar-matched for the sugar content in grapes was made by incorporating 0.68% glucose and 0.68% fructose into the base diet mouse chow. The mice were placed on the grape-supplemented diet or the sugar-matched control diet upon weaning at age 21 d and maintained on the diet for 5 wk before retinal injections until analysis at 2 wk postinjections (Fig. 1). There were a total of 26 mice in the study: There were 13 mice on each diet, and 8 mice per diet received a PQ injection and the remaining 5 mice received a saline injection.

Oxidative stress injury

Wild-type mice (male and female; strain B6;129 SF2/J; Jackson Laboratory, Bar Harbor, ME, USA) were used. Oxidative stress injury was induced in the retina at age 8 wk by subretinal injection of PQ, which has been used by many groups to simulate the elevated oxidative stress that is observed in retinal degenerations. The mice were first anesthetized by intraperitoneal injection of a xylazine-ketamine mixture and the eyes were locally anesthetized with eye drops containing 0.5% proparacaine hydrochloride (Akorn, Lake Forest, IL, USA). Mice were then subretinally injected with either 2 μL of PQ (1 mmol/L) or saline control, as described [13]. Briefly, an incision was made in the conjunctiva and the sclera, and injections were performed using a 1.5 cm 33-gauge Hamilton needle (Hamilton Company, Reno, NV, USA) between the RPE and neural retina. The left eye of each mouse was injected, and all injections were localized in the same area of the eye. All mice received the same volume of injection fluid. A successful injection was indicated by a transient retinal detachment that spontaneously resolved, which was confirmed by optical coherence tomography (OCT) analysis at 1 wk postinjection. We excluded from the study any mice with unresolved retinal detachments, bleeding, or infection.

Electroretinography

Retinal function was analyzed 2 wk after PQ injury using ERG, as described [13]. The mice were dark-adapted and then anesthetized with a ketamine-xylazine cocktail and the pupils were dilated with topical 10% phenylepherine hydrochloride. To maintain a constant body temperature, the mice were wrapped in a modified heating blanket attached to a heated running-water bath. A reference electrode was placed under the skin between the ears of the animal, a ground electrode was placed in the tail, and silver wire recording electrodes were positioned on each cornea. The mice were then placed in a Ganzfeld light-emitting chamber, and flash intensity and duration was set using the UTAS system controlled by EM for Windows software (LKC Technologies, Gaithersburg, MD, USA). The eyes were kept moist by the application of artificial tears. To measure scotopic responses, mice were exposed to single flashes of white light stimuli at intensities ranging from 1 to 100 (cd s)/m^2. To measure photopic responses, the mice were exposed to flashes of green light stimuli at intensities ranging from 0.01 to 10 (cd s)/m^2 in the presence of a dim-green-background light intensity of 1.0 (cd s)/m^2. Ten responses to each light stimulus were averaged.

Optical coherence tomography

Retinal layer thickness was measured in vivo using spectral domain optical coherence tomography (SD-OCT; Bioptigen, Research Triangle Park, NC, USA) that is optimized for rodents, as described [13]. Mice were anesthetized with ketamine-xylazine cocktail, and then pupils were dilated with topical phenylephrine (Akorn) and kept moist using artificial tears. A 1.3 × 1.3 × 1.56 mm^3 volume of the mouse retina centered on the optic disk was imaged, generating 100 b-scans. The average photoreceptor thickness was measured by segmenting the OCT images in 70 to 80 cross-sectional b-scans across the retina from the outer nuclear layer (ONL) to the inner and outer segments, inclusive, using MATLAB software and analytical programs developed by the Ophthalmic Biophysics Center at the University of Miami (OBC Segmentation).

Histology

Enucleated eyes were embedded and cryosectioned as described in [17]. Eyes were fixed in cold 4% paraformaldehyde, incubated in increasing sucrose concentration solutions, then embedded in optimal cutting temperature compound, and frozen. Eight-micrometer-thick cross sections were cut through the entire globe, and the sections were mounted onto Superfrost micro slides (VWR International, Radnor, PA, USA). The retinas were stained with 4′,6-diamidino-2-phenylindole (DAPI) to view nuclei or with hematoxylin and eosin to observe the retinal structure, and then viewed using a fluorescent microscope (Zeiss