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Original Contribution

Yttrium oxide nanoparticles prevent photoreceptor death in a light-damage model of retinal degeneration

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ABSTRACT

Photoreceptor (PR) cells are prone to accumulation of reactive oxygen species (ROS) and oxidative stress. An imbalance between the production of ROS and cellular antioxidant defenses contributes to PR degeneration and blindness in many different ocular disease states. Yttrium oxide (Y_2O_3) nanoparticles (NPs) are excellent free radical scavengers owing to their nonstoichiometric crystal defects. Here we utilize a murine light-stress model to test the efficacy of Y_2O_3 NPs (~10–14 nm in diameter) in ameliorating retinal oxidative stress-associated degeneration. Our studies demonstrate that intravitreal injections of these NPs at doses ranging from 0.1 to 5.0 μM 2 weeks before acute light stress protect PRs from degeneration. This protection is reflected both structurally (i.e., decreased light-associated thinning of the outer nuclear layer) and functionally (i.e., preservation of scotopic and photopic electroretinogram amplitudes). We also observe preservation of structure and function when NPs are delivered immediately after acute light stress, although the magnitude of the preservation is smaller, and only doses ranging from 1.0 to 5.0 μM were effective. We show that the Y_2O_3 NPs are nontoxic and well tolerated after intravitreal delivery. Our results suggest that Y_2O_3 NPs have astonishing antioxidant benefits and, with further exploration, may be an excellent strategy for the treatment of oxidative stress associated with multiple forms of retinal degeneration.

Progressive dysfunction and degeneration of photoreceptors (PRs) is a leading cause of blindness [1]. PRs experience high light exposure compared to other parts of the body and also have been shown to experience oxidative stress and accumulation of reactive oxygen species (ROS), processes that are implicated in the pathobiology of many retinal diseases, including diabetic retinopathy [2] and age-related macular degeneration (for a recent review of this literature, see [3]). Generation of ROS activates cellular antioxidant defense systems, which promote cell survival [4–6], but overproduction of ROS creates oxidative stress [6]. This can severely damage multiple cellular processes, disrupt cellular physiology, and activate apoptosis. In PRs, the high levels of polyunsaturated fatty acids (PUFAs) are particularly susceptible to lipid peroxidation [3,7,8], and oxidative damage to proteins, RNA, and DNA can also occur [4,7].

A logical way to defend against these disease processes is antioxidant therapy [9,10], and antioxidants can protect the retina and retinal pigment epithelium [11] from oxidative damage. In patients, dietary supplementation with antioxidants was effective in preventing and slowing down the progression of age-related macular degeneration [12] and delaying retinitis pigmentosa [13]. These observations suggest that delivery of highly efficacious, nonenzymatic antioxidants directly to the eye (thus avoiding issues of oral bioavailability, which have plagued other approaches) may significantly ameliorate some forms of retinal degeneration.

The transition metal yttrium (Y) has a high affinity for oxygen compared to other elements [14]. Yttrium oxide (Y_2O_3) is an important dopant for the rare earth metals and is gaining interest for application in photodynamic therapy and biological imaging [15–19]. Importantly, and in contrast to many other metals, the form of yttrium with the highest free energy is the oxide form [14], making it extremely stable. Y_2O_3 nanoparticles (NPs) are an air-stable white solid substance and are insoluble in water. A significant degree of nonstoichiometric defects occur on absorption of water and carbon dioxide from air under normal atmospheric conditions [14]. These defects are responsible for the free radical-scavenging activity of Y_2O_3 [14].

Previously, it has been reported that Y_2O_3 NPs promote survival of neuronal cells in vitro under glutamate-induced oxidative stress [14]. Endogenous ROS were quenched by Y_2O_3 NPs within 15 min

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(as measured by ROS-induced formation of the fluorescent compound dichlorofluorescein), indicating that Y_2O_3-mediated protection is due to fast-acting direct antioxidant effects, rather than indirect effects such as initiation of a complex cellular response (which occur on a longer time scale). Other studies have also shown that Y_2O_3 NPs have protective, antioxidant effects: rat pancreatic islets were protected from oxidative stress-mediated apoptosis by Y_2O_3 [20]. These antioxidant properties were comparable to those of other commonly used metal antioxidants such as ceria [14,21], which has also been shown to be effective at retarding retinal oxidative damage [21]. Here we test the hypothesis that Y_2O_3 NPs can be used to prevent oxidative retinal damage in a murine light-damage model [22,23]. Light-damage models have been widely [22–29] and successfully used to test antioxidant therapies, and our results show that Y_2O_3 NPs confer significant protection against light-induced retinal damage, suggesting that this could be an exciting approach to protecting the retina.

Materials and methods

NP characterization

Transmission electron microscopy (TEM) analysis was carried out as described [30,31] using 1 drop of a 1 mM or 5 μM Y_2O_3 NP (Sigma–Aldrich) dispersion, which was prepared under 10-W ultrasonication at room temperature.

Oxygen-radical absorbance capacity (ORAC) assay

This assay was implemented according to methods previously described [32], with some modifications. Fluorescein sodium salt (FL; 0.05–4.8 μM, Sigma–Aldrich), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH; 0.15 M, Sigma–Aldrich), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox; 5–35 μM, Sigma–Aldrich), and the Y_2O_3 NPs (5–35 μM) were all prepared in 1 × phosphate-buffered saline at pH 7.4. Initially the fluorescence of the FL was optimized within the range of 0.05–4.8 μM in 96-well plates (flat bottom, polystyrene). The fluorescence intensity was determined at 520 nm (emission) upon excitation at 485 nm using a microplate reader (FLUOstar Optima, BMG Labtech). The assay was carried out by taking 30 μl of FL (0.15 μM)+60 μl of AAPH and varying concentrations of Y_2O_3 NPs or Trolox. The NPs and AAPH were mixed in a 96-well plate first and warmed at 37 °C for 15 min and then FL was added to the mixture in the dark right before the fluorescence measurements were recorded in the microplate reader. Antioxidant capacity was determined by measuring the area under the curve of the time-dependent fluorescence intensity of FL from Y_2O_3 NPs and Trolox-treated experiments. Trolox (a vitamin E analog) was taken as a positive control for the assay. The assay was repeated four separate times and each time was performed in triplicate. To assess whether soluble metal ions released from the NPs mediate the effect, the ORAC assay was repeated with NP supernatant vs pellet. NPs (35 μM) were suspended in water in two separate tubes and they were centrifuged at 14,000 rpm for 30 min to pellet the particles. The ORAC experiment was carried out in the same way as above with the supernatant vs NP pellet along with the respective controls.

Injections and light exposure protocols

Albino mice (Balb/C) were bred in-house and were maintained in the breeding colony under cyclic light (30 lx, 12 L:12D) throughout the study except when specified in the light-exposure paradigm. The University of Oklahoma Health Sciences Center Institutional Animal Care and Use Committee approved all experiments and animal care, and all animal experiments complied with guidelines set forth by the Association of Research in Vision and Ophthalmology. For light-exposure experiments, animals were dark-adapted for 48 h. After the dark adaptation the mice (two per cage) were placed in a transparent polycarbonate cage, which had the food in the bottom of the cage and a water bottle placed at the side of the cage to ensure even light penetration. This cage was then placed in a light box for 2 h with constant bright light at the specified intensity. Upon completion of the light exposure, the mice were returned to their normal cages and replaced in their regular housing area under normal cyclic light (30 lx, 12 L:12D) for the duration of the study. Light exposure and injections were carried out at the same time of day throughout the study. Transscleral intravitreal injections of 2 μl of NP suspension were performed as described previously [33].

Electroretinography

Full-field electroretinography was performed as described previously [33]. Maximum scotopic and photopic A- and B-wave amplitudes were plotted (n=8–12).

Morphometric analysis

Eyes were enucleated, dissected, and fixed as described previously [34]. Hematoxylin and eosin (H&E)-stained sections from each eye along the vertical meridian were used to measure outer nuclear layer (ONL) thickness and the number of ONL nuclei using ImageJ (U.S. National Institutes of Health) [34]. Images for morphometry were collected from at least three eyes per group, starting from the optic nerve head (ONH) and proceeding toward the periphery at 435-μm intervals.

Statistical analyses

The standard deviation (SD) was used for the statistical analysis of area under the curve measurements from triplicates. One- or two-way ANOVA with Bonferroni's post hoc comparison was used for all other statistical analysis. Significance was defined as $P < 0.05$, and data in all other figures are presented as the mean ± SEM.

Results

Nanoparticle characterization

We first undertook a physical characterization of the Y_2O_3 NP suspension. High-resolution TEM of Y_2O_3 NPs at 1 mM demonstrated that the individual NPs were monodisperse and spherical in shape (arrows, Fig. 1A, left), although at this high concentration they tended to aggregate. To confirm that the particle shape was retained at our working concentrations, we also conducted TEM on particles at 5 μM (Fig. 1A, right). Whereas the particles were significantly rarer on the grid because of the lower concentration, they exhibited the same shape and size characteristics (inset in Fig. 1A, right, shows larger version of particle with arrowhead). Measurements from the TEM indicated that the particles were ~10–14 nm in diameter. The shape was as expected, and previously it has been demonstrated that spherical Y_2O_3 NPs possess less cellular toxicity than other shapes [17]. For subsequent studies we tested the efficacy of these NPs at 0.1, 1.0, and 5.0 μM, as similar doses have been tested for other nonenzymatic antioxidants [21].

To assess the direct free radical-scavenging activity of the Y_2O_3 NPs, we used the standard ORAC assay in which the ability of antioxidants to suppress AAPH-derived free radicals is measured by

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