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The therapeutic potential of sulforaphane on light-induced photoreceptor degeneration through antiapoptosis and antioxidant protection

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ABSTRACT

Oxidative stress due to excessive light exposure can exacerbate a variety of human retinal diseases by accelerating photoreceptor cell death. The thioredoxin (Trx) system is considered to play a crucial role in reduction/oxidation (redox) regulation of signal transduction and in cell defense against oxidative stresses. Sulforaphane (SF) protects cells from oxidative damage through nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is responsible for multiple detoxification processes, including elevating the expression of Trx. This study sought to demonstrate whether SF increased Trx expression in retinal tissues *in vivo* and whether it could preserve the photoreceptors from degeneration induced by oxidative stress. Our data clearly showed that pretreatment with SF abated photoreceptor cell loss, in association with increased expression of Nrf2 and Trx, subsequently activating the Ras/Raf1/Erk signaling pathway and decreasing the expression of Bak1, Cyt-c release and the activity of caspase-3 in light-induced mouse retinas. These data suggested that the therapeutic potential of SF in retinal degeneration due to oxidative stress might partially involve anti-caspase and antioxidant protection mediated by Trx.

1. Introduction

Photoreceptor loss is the leading cause of many degenerative diseases of the human retina, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). Light-induced retinal degeneration and the death of photoreceptor cells have been investigated under experimental conditions to model the progression of the different pathologies of these retina degenerative diseases (Paskowitz et al., 2006; Rutar et al., 2010). The factors that influence light-induced photoreceptor cell death include different light sources, environmental insults, age, body temperature, circadian rhythms and genetic backgrounds (Organisciak et al., 2000; Hunter et al., 2012). Previous studies have demonstrated that exposure to excessive levels of white light induced photoreceptor degeneration, and the apoptotic pathway was the main route of light-induced photoreceptor cell death (Venugopal and Jaiswal, 1998; Wenzel et al., 2005), including the activation of AP-1, excessive influx of calcium ions, and damage to the mitochondria and DNA (Sasaki et al., 2011). Photooxidative stress has been implicated as a mechanism of retinal light damage. Maintenance of photoreceptor cell integrity against photo-oxidative insult is mediated by endogenous antioxidant systems (Ohira et al., 2003) and exogenous antioxidants (Chen et al., 2004). However, the exact mechanisms remain unclear.

Sulforaphane (4-methylsulfinyl-*n*-butyl isothiocyanate) belongs to a group of phytochemicals or disease-fighting compounds known as the isothiocyanates, which occur in cruciferous vegetables, such as broccoli. Several studies have shown that SF protected against oxidative stress-induced tissue damage, including damage to photoreceptors, lens cells and retinal pigment epithelial cells, due to its anti-oxidant and anti-inflammatory properties (Ambrecht et al., 2015; Ye et al., 2013; Liu et al., 2013). The major

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mechanism by which SF protects cells was traditionally believed to be by altering the expression of phase 1 and phase 2 metabolizing enzymes. SF activates phase 2 enzymes via nuclear factor (erythroid-derived 2)-like 2 (Nrf2), which is responsible for multiple detoxification processes, including regulating the expression of thioredoxin (Trx) and thioredoxin reductase (TrxR), as well as other antioxidants, and raising cell defenses against oxidative damage (Talalay et al., 2003; Zhang et al., 2003). Our previous study showed that SF treatment significantly delayed cochlear degeneration by enhancing extracellular signal-regulated kinase (Erks) activation and subsequently up-regulating Nrf2 and cis-acting antioxidant response element (ARE)-regulated genes, including the Trx/TrxR redox system, in tubby mutation mice (Kong et al., 2009). Furthermore, it is becoming increasingly clear that there are multiple mechanisms activated in response to SF, and these mechanisms seem to have some degree of interaction in synergistically affording chemoprevention (Juge et al., 2007).

The use of inducers has been considered less invasive and safer than intraocular injection of some proteins or gene transfection to protect the retina from degeneration *in vivo*. Thus, the purpose of this study was to test whether SF induced Trx in retinal tissues *in vivo* and whether pretreatment with SF had any effects against retinal light-induced damage. Our data clearly showed that pretreatment with SF inhibited photoreceptor degeneration by inducing Trx, subsequently reducing oxidative stress and regulating apoptosis in light-exposed mouse retinas.

2. Materials and methods

2.1. Animals

BALB/cJ mice between two and three months of age were used for the experiments. All of the procedures were approved by the Institutional Animal Care and Use Committee of the Dalian Medical University in Dalian, China.

2.2. Intracardiac injection and light-induced photoreceptor degeneration

For intracardiac pretreatment, the mice were treated with SF (S8046, LKT Laboratories Inc., St. Paul, MN, USA) once per day for 7 days and then were exposed to light after the final treatment with SF (18 mg/kg, Light + SFI; or 25 mg/kg, Light + SFII). Vehicle animals were injected with phosphate-buffered saline (PBS, Light+) intracardially. Control animals were not exposed to light and received no injections (Control). The procedure for exposure to light was the same as previously described (Hafezi et al., 1997; Tanito et al., 2002a). In brief, the mice were dark adapted for 24 h before the experiments and were exposed to 5000 lux of diffuse, cool, white fluorescent light for 24, 48 or 72 h to determine an acute light-induced retinal degeneration model for subsequent experiments to detect the protective effect of SF. During light exposure, the mice were maintained in transparent polycarbonate cages (two or three mice per cage) with stainless-steel wire covers. A water bottle was kept at the side of the cage, and food was placed in the bottom of the cage on the bedding. The temperature during exposure to light was maintained at 25 ± 1.5 °C. The eyes were enucleated 4 days after light exposure for outer nuclear layer (ONL) thickness analysis (Ueki et al., 2008) and electroretinography. The eyes were immediately enucleated after light exposure to detect the possible rapid changes via western blot analysis, semi-quantitative RT-PCR, immunochemistry and the remaining procedures. Except the animals were killed by perfusion with paraformaldehyde for preparation of retinal tissue sections, euthanasia by CO_2 inhalation was performed in the other procedures.

2.3. Electroretinography (ERG)

Briefly, after the animals were anesthetized and their pupils were dilated 4 days after light exposure, full-field ERG was performed using an LKC electroretinogram system (GT-2008V-3, Gotec, Inc., Chongqing, China), as described previously (Kong et al., 2007). Five responses were averaged with flash intervals of 20 s. For quantitative analysis, the mean A- and B-wave amplitudes were recorded and compared from PBS- and SF-treated mice.

2.4. Preparation of retinal tissue sections

The mice were perfused through the left cardiac ventricle with phosphate-buffered saline (PBS) and then were perfused with freshly prepared 4% paraformaldehyde containing 0.25% glutaraldehyde in PBS. Their eyes were removed and embedded in paraffin. Sagittal sections containing the whole retina, including the optic disc, were cut to 5 μm in thickness.

2.5. Morphological analysis by quantitative histology

The retinal sections obtained were stained with hematoxylin-eosin (H&E). Two sections from each eye were analyzed. In each section, digitized color images from four locations, two from the superior retina (100–800 μm above the optic disc) and two from the inferior retina (100–800 μm below the optic disc), were obtained with a digital imaging system (Olympus, Tokyo, Japan). The obtained images were opened on a computer display. In each of the superior and inferior hemispheres, the ONL thickness was measured at defined points. Each point was centered on adjacent 220 μm lengths of retina. The first point of measurement was taken at approximately 220 μm from the optic nerve head, and subsequent points were located more peripherally. The data represent the average of 4 animals from each group.

2.6. TdT-mediated dUTP nick-end labeling assay (TUNEL assay)

Detection of apoptosis by TUNEL assay was performed using a commercially available *in situ* apoptosis detection kit (Biovision, Mountain View, CA, USA) on sections obtained according to the manufacturer's protocol. Observation of TUNEL-positive cells and imaging were conducted using a Nikon Eclipse 800 microscope on three eyes from each group, and representative images are shown. Four animals were used in each group.

2.7. DNA ladder assay

Agarose gel electrophoresis was used to detect DNA laddering by the method of Sorenson et al. (Sorenson et al., 1990). Briefly, retina tissues were obtained from each group and were pulverized in lysis buffer including10 mM Tris-HCl (pH 8.0), 100 mM EDTA (pH 8.0), and 0.5% SDS. Then, RNase A was added to a final concentration of 20 μg/ml, followed by incubation at 37 °C for 30 min. Then, proteinase K was added to a final concentration of 100 μg/ml, followed by incubation at 55 °C overnight. In addition, an equal volume of phenol was added and gently extracted by inverting the tube several times, and the mixture was centrifuged at 800*g* for 10 min. Finally, the aqueous phase was removed, and an equal volume of phenol/chloroform/isoamyl alcohol mixture (25:24:1) was added and centrifuged at 800*g* for 10 min. The products were resolved on 1% agarose gels running at 90 V for 40 min and were stained with ethidium bromide (EB).