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Protection of retinal function by sulforaphane following retinal ischemic injury

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ABSTRACT

Sulforaphane, a precursor of glucosinolate in cruciferous vegetables such as broccoli and cauliflower, has been shown to protect brain ischemic injury. In this study, we examined the effect of systemic administration of sulforaphane on retinal ischemic reperfusion injury. Intraocular pressure was elevated in two groups of C57BL/6 mice (n = 8 per group) for 45 min to induce retinal ischemic reperfusion injury. Following retinal ischemic reperfusion injury, vehicle (1% DMSO saline) or sulforaphane (25 mg/kg/day) was administered intraperitoneally daily for 5 days. Scotopic electroretinography (ERG) was used to quantify retinal function prior to and one-week after retinal ischemic insult. Retinal morphology was examined one week after ischemic insult. Following ischemic reperfusion injury, ERG a- and b-wave amplitudes were significantly reduced in the control mice. Sulforaphane treatment significantly attenuated ischemic-induced loss of retinal function as compared to vehicle treated mice. In vehicle treated mice, ischemic reperfusion injury produced marked thinning of the inner retinal layers, but the thinning of the inner retinal layers appeared significantly less with sulforaphane treatment. Thus, sulforaphane may be beneficial in the treatment of retinal disorders with ischemic reperfusion injury.

Retina ischemia is a leading cause of low vision in the United States. It is a major contributor to tissue damage in diseases including acute angle-closure glaucoma, diabetic retinopathy, retinal vascular occlusions and retinopathy of prematurity (Osborne et al., 2004). Retinal ischemic injury disrupts retinal blood flow and results in retinal oxygen and other nutrient deficiencies; however, the subsequent reperfusion exacerbates the tissue damage by the generation of reactive oxygen species (Wei et al., 2011). The key elements of the pathologic alteration in retinal ischemic reperfusion injury are the generation of reactive oxygen species (ROS), apoptosis and inflammation during the reperfusion (Osborne et al., 2004). ROS produced during normal cellular respiration are normally neutralized by cellular antioxidant defenses before these free radicals have the opportunity to damage the cells. Modulation of oxidative stress has been shown to reduce histopathologic changes in retinal ischemic reperfusion injury (Chen et al., 2009).

Sulforaphane (SFN), a naturally occurring dietary isothiocyanate, is a precursor of glucosinolate in cruciferous vegetables such as broccoli and cauliflower (Zhang et al., 1992). SFN has been widely studied for its anti-carcinogenic, anti-inflammatory, anti-apoptotic and anti-oxidative functions in different tissues (Angeloni et al., 2009; Baek et al., 2008; Yoon et al., 2008; Zhao et al., 2006). SFN has been shown to protect the kidneys, heart, brain, and liver against ischemic injury through the activation of the Nrf2 antioxidant response element pathway (Piao et al., 2010; Ping et al., 2010; Yoon et al., 2008; Zhao et al., 2010). SFN upregulates thioredoxin in retinal tissue and mediates cytoprotection against light-induced photoreceptor and retinal pigment epithelial cell damage in mice (Tanito et al., 2005). Recently, it is reported that pre-treatment with SFN also diminishes retinal ganglion cell apoptosis and protects against thinning of the inner retinal layer upon retinal ischemic injury (Pan et al., 2014).

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The purpose of this study was to determine whether SFN can treat retinal ischemic injury in mice as quantified structurally, with changes in retinal morphology, and functionally, with changes in electroretinographic (ERG) responses.

C57BL/6 mice (purchased from Jackson Laboratory) were used for this study. 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. C57BL/6 mice (6–8 weeks old) were randomly assigned to two groups: SFN-treated retinal ischemic injury mice and vehicle-treated retinal ischemic injury mice. Anesthesia was administered using ketamine (100 mg/kg body weight) and xylazine (5 mg/kg body weight) and topical 0.5% proparacaine hydrochloride (Alcaine, Alcon) was applied to the eyes of the mice prior to the all procedures. Retinal ischemic reperfusion injury was induced by transient elevation of intraocular pressure for 45 min as previously described (Vin et al., 2013). The anterior chamber of the right eye of each mouse was cannulated with a 30-gauge infusion needle connected to an elevated physiological saline bag. The intraocular pressure was raised to 70–80 mm Hg for 45 min. Rapid blanching of the ocular fundus and the collapse of the retinal arteries was observed using an indirect ophthalmoscope, verifying retinal ischemia. The other eye of the same mouse was used as the non-ischemia control and was not cannulated with the infusion needle. The non-ischemia control group was comprised of SFN and vehicle-treated animals as the results are comparable. Following ischemic insult, vehicle (1% DMSO saline) or SFN (25 mg/kg/day, LKT Laboratories, Inc.) was administered intraperitoneally daily for 5 days. The effective dose (25 mg/kg/day) of SFN was determined in preliminary experiments in which several SFN doses 5–50 mg/kg/day were tested for retinal function changes. This was based on doses shown to protect against brain ischemic injury and brain inflammation (Innamorato et al., 2008; Zhao et al., 2006). SFN was prepared in 1% DMSO saline solution prior to use. Since the retina clearly showed functional and structural damage seven days after ischemic insult in our previously published experiments (Bu et al., 2010; Vin et al., 2013), we treated the mice with SFN for five days and then determined the functional and structural damage at day seven.

Retinal function was evaluated prior to inducing ischemic retinal injury as well as seven days after the injury. Mice were dark-adapted overnight. They were then anesthetized and their pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride (Bausch & Lomb Inc.). Using a stainless steel electrode coated with 1% methylcellulose, the ERG was recorded from the corneal surface with a series of stimulus luminances. Needle electrodes were subcutaneously inserted in the cheek and the tail served as reference and ground leads, respectively. The ERG responses were differentially amplified (0.3–1500 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. At 7.5 ms after the flash onset from the pre-stimulus baseline, the a-wave amplitude was measured. The amplitude of the b-wave was measured from the a-wave trough to the peak of the b-wave. If no a-wave was present,

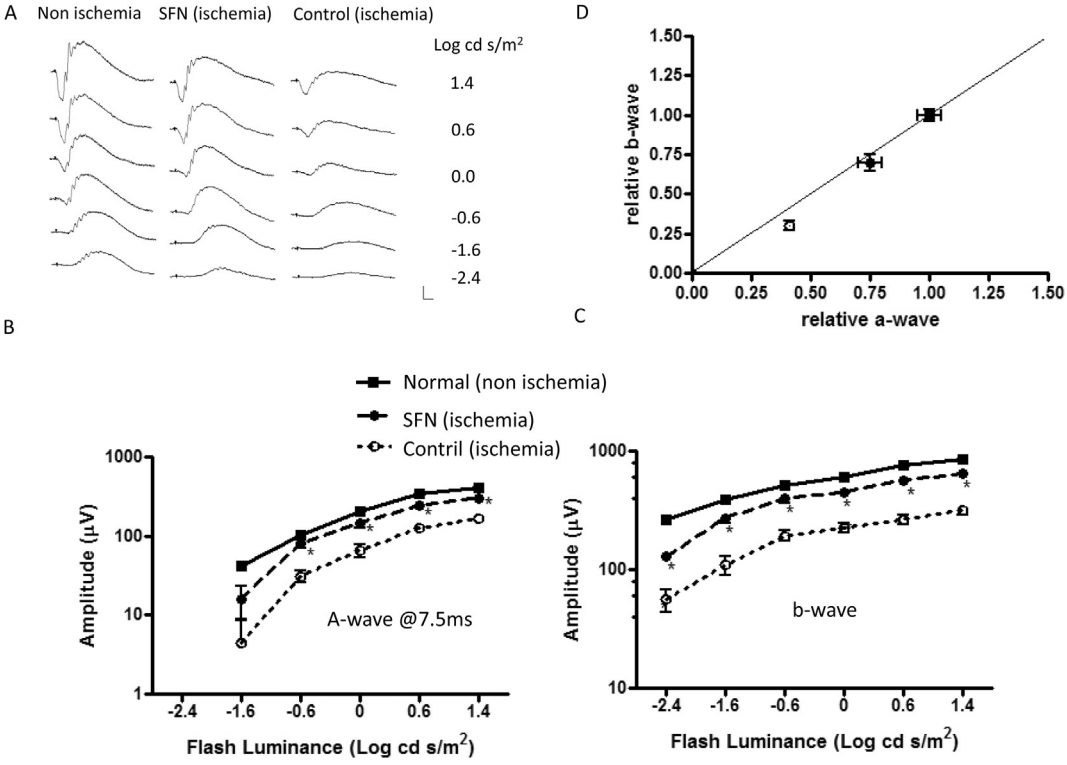

Fig. 1. Effect of sulforaphone on retinal function post-ischemic reperfusion injury. (A) Dark-adapted ERG waveforms from 6 representative flash luminance in normal (non-ischemic), SFN-treated (ischemic) and control-treated (ischemic) eye. Scales bar, 250 μV and 20 ms. Luminance-response functions for (B) a-wave and (C) b-wave components of the ERG. (D) Relative changes in a- and b-wave amplitudes observed in normal (non-ischemic), SFN-treated (ischemic) and control-treated (ischemic) mice. Data are plotted relative to the average of the normal (non-ischemic) mice. The diagonal line indicates an equivalent reduction in a- and b-wave amplitudes. Data points indicate the means ± SD (n = 8–16) from normal (non-ischemic), SFN-treated and vehicle-treated, SFN-treated (ischemic) and control-treated (ischemic) eye. *p < 0.01; two-way ANOVA with Bonferroni post hoc analysis comparing vehicle-with SFN treatment.