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NEUROGLOBIN – A POTENTIAL BIOLOGICAL MARKER OF RETINAL DAMAGE INDUCED BY LED LIGHT

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Abstract—Neuroglobin (NGB), a protein highly expressed in the retina, has been shown to be up-regulated to protect neurons from hypoxic and ischemic injuries. It exhibits neuroprotective functions and plays an important role in the survival of neurons. Recent studies show that light-emitting diode (LED) white light emitted significant amounts of blue light (short-wavelength), which may be harmful to retinal cells, but the studies about biomarkers for evaluating the damage from LED white light are still insufficient. In our study, we found that NGB levels in the retina showed a two-fold increase and peaked at 1 h after a 1-h exposure to blue light (453 nm) which did not cause damage to the retina. However, retinal damage was observed after 2 h of blue-light irradiation, which induced an approximate sevenfold increase of NGB levels as confirmed by Western blot and RT-PCR analysis. Immunofluorescence study demonstrated that NGB was predominantly up-regulated in the ganglion cell layer (GCL), plexiform layer (PL) and photoreceptor layer (PRL). We also examined *Ngb* mRNA and protein expression in the damaged retina induced by light of other wavelengths given equal photon fluxes. The LED red light (625 nm), green light (527 nm) and blue light (453 nm) increased the expression of NGB and caused TdT-mediated dUTP nick-end labeling-positive cells, especially in the blue-light group. In addition, a negative correlation between NGB and rhodopsin was observed. These findings suggested that there was a correlation between NGB expression and the severity of the retinal damage, indicating NGB's potential function as a biological marker of retinal damage induced by LED light. **© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: neuroglobin, retinal light damage, LED light, biological marker.

INTRODUCTION

Light-emitting diode (LED) is a solid-state semiconductor converting electrical energy directly into visible light. Unlike tungsten lamp, LED lights project light by stimulating phosphors with blue light, thus they contain an extremely high content of hazardous constituents of visible spectrum (Behar-Cohen et al., 2011). Since shorter wavelengths of approximately 400–500 nm are particularly more hazardous, LED light may be more likely to cause photochemical damage to retinas than any other light source (Okuno et al., 2002).

However, the hypothesis about retinal light damage remains unclear. There are few researches regarding the biological marker. The current recognized biomarker is rhodopsin, which is established based on the quantitative experimental results of the photochemical damage to the retina, especially the study on blue light-induced retinal degeneration mediated by rhodopsin (Grimm et al., 2000; Organisciak and Vaughan, 2010). When both metabolic rhodopsin regeneration and photoreversal of bleaching were inhibited, blue-light exposure caused only a modicum of lesions. No damage occurred in the retina without rhodopsin (Grimm et al., 2000). Another mechanism of photochemical damage to retina was through oxidative stress. Blue-light irradiation significantly increases the production of mitochondrial superoxide radicals in retinal cells (Knels et al., 2011). Some researchers suggest that the bleaching of rhodopsin caused damage through oxidative stress (Delmelle, 1978; Rozanowska et al., 1998). Nevertheless, rhodopsin is unstable and the concentration may be too low for it to be the photodynamic sensitizer (Noell, 1980).

Neuroglobin (NGB) is a protein discovered in the brain (Burmester et al., 2000). It displays a typical globin fold with a high affinity to oxygen (Pesce et al., 2003; Vallone et al., 2004), and is widely and specifically expressed in the neurons of central and peripheral nervous systems of vertebrates (Reuss et al., 2002; Wystub et al., 2003). The structural features, ligand-binding properties and neural expression of NGB suggest

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCL, ganglion cell layer; LED, light-emitting diode; NGB, Neuroglobin; OCT, optimum cutting temperature compound; PBS, phosphate-buffered saline; PL, plexiform layer; PRL, photoreceptor layer; RIPA, radioimmunoprecipitation assay; TBS, Tris-buffered saline; TUNEL, TdT-mediated dUTP nick-end labeling.

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it plays a role in oxygen homeostasis of neuronal tissues (Moens and Dewilde, 2000; Dewilde et al., 2001). Majority of the works have been concentrated on investigating the functions and mechanisms of NGB through gene expression alteration approaches, which confirm that the overexpression of NGB confers neuroprotection against neuronal hypoxia or ischemia-induced damage in cultured neurons and in cerebral ischemic animal models (Sun et al., 2001; Khan et al., 2006; Fordel et al., 2007; Li et al., 2008; Li et al., 2010).

The estimated concentration of NGB in the retina is about 100-fold higher than that in the brain and is in the same range as that of myoglobin in the muscle (Schmidt et al., 2003). *Ngb* mRNA is detected in the perikarya of the nuclear and ganglion layers of the neuronal retina, whereas the protein is present mainly in the plexiform layers (PLs) and in the ellipsoid region of photoreceptor inner segment (Schmidt et al., 2005). The distinctive distribution correlates with the subcellular localization of the mitochondria and the relative oxygen demands (Bentmann et al., 2005). Since visual performance of the eyes requires large amounts of oxygen for the maintenance of phototransduction mediated by rhodopsin and the transmission at the synapses (Anderson and Saltzman, 1964; Pugh and Lamb,2000). NGB is more likely to play a critical role in visual physiological cycle, although the multiple feedback loops remain unclear. Studies on the expression of NGB in the retina are still at the initial stage. However, emerging reports have revealed that NGB presents an underlying protection against oxidative stress and anti-apoptotic effect in the retina. A study examines NGB in a model of glaucoma and the results indicate that NGB increased the resistance of retinal cells to oxidative condition and the overexpression of NGB reduces intraocular pressure-associated superoxide production (Wei et al., 2011). NGB also increases rapidly in ocular hypertension-induced acute hypoxic–ischemic retinal injury (Shi et al., 2011). It seems that NGB acts as an indicator for disease in early stage. Another investigation showed that the overexpression of NGB is associated with decreased mitochondrial DNA damage, significant preservation of retinal thickness, less activated caspase 3 protein expression and apoptosis in *Ngb*-Tg mice (Chan et al., 2012). Lechauve et al. show that the *in vivo* knockdown of *Ngb* expression by subjecting rats' eyes to electroporation causes deleterious effects on retinal structure and function including decreasing nerve fiber density and impairment of visual function (Lechauve et al., 2012). Other findings have suggested several possible neuroprotective roles of NGB including oxygen sensing, modulation of cell signaling pathways and maintenance of mitochondria function (Kriegl et al., 2002; Wakasugi et al., 2003; Burmester et al., 2007; Khan et al., 2008; Liu et al., 2009). According to the above mechanisms that involve retinal light damage and the similarity between retinal neurocytes and neurons in the brain, we hypothesized that NGB might be a potential marker for retinal light damage.

In this study, we clarified whether or not NGB might be involved in LED light-mediated retinal damage, and focused on raising public awareness of the relationship between NGB and photochemical damage induced by LED light.

EXPERIMENTAL PROCEDURES

Animals

All experiments conformed to the ARVO statement regarding the use of animals in ophthalmic and vision research. In addition, all efforts were made to minimize the number of animals used for the research and their suffering. Male or female Sprague–Dawley rats (SPF Laboratorial Animal Center, Sun Yat-sen University, Guangzhou, China), each weighing between 180 g and 200 g, were exposed to 60-lux cyclic light (light/dark, 12 h/12 h) for 3–7 weeks. Later on, the rats were left overnight (16 h) in the dark and were anesthetized with 10% chloral hydrate (0.3 ml/100 g) prior to light treatment. And keeping the rats anesthetized during light treatment. All rats (including the experimental group and the control group) were treated with atropine eye drops for mydriasis before irradiation.

Lighting treatment

The Light Application Set-up contained two parts, the Light Panel (Joinmax Display Technology Co., Ltd. in Guangzhou, China (Fig. 1A) and the light box. LED lights (LE RTDUW S2 W, OSRAM, Germany) were placed in the Light Panel (Fig. 1A, B), which was located on the top of the light box (Fig. 1C). Two to four rats were placed at the bottom of the light box to undergo light treatment for each experiment, and there was a vent used to ventilate (Fig. 1C). An optical power meter (LP-3A, Physcience Opto-Electronics, Beijing, China) was used to measure the average luminous power on the floor of the lighting box and the Spectrometer (USB 4000, Ocean Optics, Shanghai, China) was used to measure the light spectrum. The Light Application Set-up was connected to a computer through a regulator. Therefore, after we installed the software in a computer, we adjusted the wavelengths of the LED lights and the duration of exposure in the lighting box.

Different light intensities gave equal photon fluxes around each cornea (2.26×10^{19}/s·m^2). These intensities of exposure were at 10 W/m^2 for 453-nm blue light, 8.6 W/m^2 for 527-nm green light and 7.0 W/m^2 for 625-nm red light. Using an optical power meter to establish the lighting parameter and before each experiment, resetting the parameter with the software. The light box was placed in a darkroom during lighting treatment, and the temperature and humidity were controlled.

Current evidence supports the theory that photochemical injury requires a threshold radiant exposure dose of approximately 2.2×10^5 J/m^2 at 446 nm (CIE, 2000). The 1-h exposure time for exploring the expression of NGB in Fig. 2 was referred to the above threshold radiant exposure dose, which focused on using a low-radiant exposure dose that would not induce retinal damage. In Figs. 3–5, the rats were exposed for 2 h to

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