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Protective effect of crocin against the declining of high spatial frequency-based visual performance in mice

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ABSTRACT

The high spatial frequency-based vision was primary on cone rich macular vision. Crocin is a major bioactive constituent of saffron (*Crocus sativus* L. flowers) and gardenia fruit (*Gardenia* spp.), which has powerful anti-oxidant capacities in retina. The study aim to evaluate the influence of crocin on visual performance in light-damaged mouse retinas in vivo. The visual acuity (VA), and visual contrast sensitivity function (VCSF) were measured by the behavioral optomotor reflex method. The histological changes of retinas were monitored. Both VA and VCSF declines, and that accompanied by Müller cell hypertrophic gliosis and cone cellular damage were detected in the vehicle groups. Prophylactic crocin treated groups improved VA and VCSF on sensitive to high spatial frequency, protected retinal integrity and Müller cells, and modulated the cellular function of cone. This study provides the potential use of crocin to ameliorate the visual performance due to its anti-photodamage and cytoprotective effects.

1. Introduction

High-energy visible light may have an impact on the incidence of retinal disorders (Margrain, Boulton, Marshall, & Sliney, 2004). Focal light-emitting diode (LED)-induced retinal phototoxicity exacerbates retinal photoreceptor cell dysfunction (Krigel et al., 2016; Margrain et al., 2004). Short wavelengths are linked to dysfunction of visual phototransduction (Chen, Yoshida, & Bitensky, 2008; Lobanova et al., 2010), aberrant rhodopsin localization (Grimm et al., 2001; Nakamura, Kuse, Tsuruma, Shimazawa, & Hara, 2017), lipofuscin-laden cell death (Brown, 1991; Sparrow, Nakanishi, & Parish, 2000) and sustained inflammatory retinal injury (Algvere, Marshall, & Seregard, 2006; Chang, Kim, Kim, Park, & Kim, 2016). These are regarded as the predominant risk factors accelerating retinal ageing and vision problems. Loss of visual performance, including visual acuity (VA) and visual contrast sensitivity function (VCSF), usually occur prior to early-stage retinopathy or accompanied by photoreceptor damage (Owsley, 2003; Thayaparan, Crossland, & Rubin, 2007). Declines in VA and VCSF may aggravate with disease progress (Bellmann, Unnebrink, Rubin, Miller, & Holz, 2003; Owsley, 2003; Peyrin, Ramanoel, Roux-Sibilon, Chokron, & Hera, 2017).

Mammalian photoreceptors are first-order visual pathway neurons. They consist of rods and cones. Rod-based vision is specialised to expand light sensitivity at the expense of resolution in dim lighting

Abbreviations: VA, visual acuity; VCSF, visual contrast sensitivity function; LED, light-emitting diode; OKR, optokinetic reflex; fERG, flash electroretinography; AMD, age-related macular degeneration; cpd, cycle per degree; ONH, optic nerve head; ONL, outer nuclear layers; OS-IS, outer segments-inner segments; INL, inner nuclear layer

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conditions. Bright light-sensitive cones function are essential for macular-, colour-, and high spatial frequency-based vision processing (edges, details, sharp transitions). Clinically, the declining of macular vision or loss of high spatial frequency-based vision was found to associate with cone dysfunction. Additionally, the Müller cell dysfunction or loss of its related cone-specific visual cycle might also be responsible for early macular vision impairment (Bringmann et al., 2006; Sahu & Maeda, 2016; Scholl, Kirchhof, & Augustin, 2010). Certain bioactive aldehydes generated by retinal photo-oxidative stress may damage and cause dysfunction in photoreceptors (Song, Song, Wang, Li, & Dunaief, 2016; Tanito, Elliott, Kotake, & Anderson, 2005). In contrast, some natural supplements have been shown to attenuate the oxidative damage and reverse retinal cell degeneration in vivo (Bian et al., 2017; Fang et al., 2016; Laabich et al., 2006). Nonetheless, their influences on visual performance, spatial frequency-based vision processing, and the extent of specialised cellular damage have not yet been systematically characterised.

Crocin (chemical formula $C_{44}H_{64}O_{24}$) is a major pharmacological component extracted from saffron or gardenia fruit decoctions (Ishizuka et al., 2013; Khazdair, Boskabady, Hosseini, Rezaee, & Tsatsakis, 2015). It has been used in the treatment of several neurovascular disorders and ameliorates inflammation, depression, coronary artery diseases, and cerebrovascular diseases (Christodoulou, Kadoglou, Kostomitsopoulos, & Valsami, 2015; Khazdair et al., 2015; Shafiee, Arekhi, Omranzadeh, & Sahebkar, 2017; Vakili, Einali, & Bandegi, 2014). Clinical ophthalmological studies have shown that crocin can improve ocular circulation (Xuan, Zhou, Li, Min, & Chiou, 1999) and retinal flicker sensitivity threshold (Falsini et al., 2010). *In vivo* animal studies have indicated that crocin and its deglycosylated form crocetin are effective against stress-mediated retinal cell degeneration (Chen, Qi, & Yang, 2015; Ishizuka et al., 2013; Laabich et al., 2006; Qi et al., 2013; Yamauchi et al., 2011). It has been reported that crocin could interfere with the PI3K/AKT (Qi et al., 2013), ERK (Chen et al., 2015; Ishizuka et al., 2013), and NF-κB signalling pathways (Ishizuka et al., 2013) and upregulate cellular redox reactions (Chen et al., 2015). Nevertheless, the in vivo effects of crocin on functional of visual performance and photoreceptor are not yet known.

Despite the distribution and composition of the photoreceptor is different between the human and rodents retina. Human retina has cone-rich macula (fovea). The rodents (laboratory mice and rats) do not have macula (fovea), which retina is rod-dominated with a fewer and non-centralized of cone photoreceptor. A clinical study reported that short-term saffron supplementation may improve retinal flicker sensitivity and increase macular region-based flash electroretinography (fERG) amplitude in patients with early age-related macular degeneration (AMD) (Falsini et al., 2010). Similarly, studies indicated that administration of crocin or crocetin was found to ameliorate ERG amplitude expression in various mouse retinopathy models (Ishizuka et al., 2013; Ohno et al., 2012; Yamauchi et al., 2011). Visual performance depends on the integrated visual processing pathway in which primary retinal image information is transferred from the photoreceptor cells and converted into electrical signals via the optic nerve to visual cortex. Whilst ERG amplitude recordings indicate the local changes in electrical impulse occurring within the neural retina, they do not predict simple visual features, especially the high spatial frequency-based vision (edges, details, sharp transitions) (Williams & Jacobs, 2007). Alternatively, the optokinetic reflex (OKR) is a useful tool for determining integral visual behaviour from the primary retinal image to secondary visual processing in visual cortex. OKR have used to observe the visual performance in animal models of retinal disease, VA and VCFS thresholds were dependent on residual function of photoreceptor (Kretschmer, Sajgo, Kretschmer, & Badea, 2015; Prusky, Alam, Beekman, & Douglas, 2004). The aim of this study was to investigate the protective efficacy of crocin on visual performance by ameliorate photoreceptor cellular function in light-damaged mouse retinas. It is known that retinal abnormal changes originate primarily with oxidative stress after light exposure (Osada et al., 2017; Yamauchi et al., 2011). The acute light exposure mediated oxidative damage in retinas can be ameliorated by crocin treatment (Yamauchi et al., 2011). In presented study, the extent to which visual performance compensated by enhancing of residual photoreceptors underlying the effects of crocin was additionally evaluated.

2. Material and methods

2.1. Animals and experimental design

Female CD-1® (ICR) mice were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan), and assigned either to one blank control group (n = 8) or one of three cyclic light-emitting diode (LED) light exposure groups (600–1000 lx, 12 h:12 h light–dark cycle): (1) vehicle-treatment group (n = 9), (2) prophylactic treatment with crocin 0.25 mg kg^{-1} body weight (LED-crocin 0.25 mg kg^{-1}, n = 7), and (3) prophylactic treatment with crocin 5 mg kg^{-1} body weight (LED-crocin 5 mg kg^{-1}, n = 7). Light damage of the experimental mice was induced by exposure of LED light for 30 days, 12 h of light exposure condition with 600 ~ 1000 lx and 12 h of dark condition with < 1 lx. Mice stayed in the dim condition (50 ± 10 lx of illuminance) before visual function analysis or sacrifice. Crocin (Crocin-1) was purchased from Santa Cruz Biotechnology Inc. (sc-217957, Santa Cruz, CA, USA) (Fig. 1A). Crocin was administered by gavage at 0.25 mg kg^{-1} or 5 mg kg^{-1} twice daily from day 0 (1 d before LED light exposure) to day 35 (Fig. 1B). All animal experimentation was approved by the Chung Shan Medical University Institutional Animal Care and Use Committee and conducted in accordance with guidelines for the use of animals in ophthalmic and vision research.

2.2. Determination of thresholds of visual acuity (VA) and visual contrast sensitivity (VCSF)

The VA and VCSF tests are based on animal optomotor reflex. The thresholds are determined from reflexive head movements occurring when stimulus grating is displayed in the visual fields of the mice (Prusky et al., 2004; Umino, Solessio, & Barlow, 2008). In the VA and VCSF tests, the mice were placed on an elevated stand in front of a monitor screen. The distance between the monitor and the animal's eyes was 15 cm covering 110 by 90 degrees of visual field. The monitor screen displayed equal width and interval vertical gratings to excite the optomotor reflex of the mice. Once the vertical grating started to display, the reflexive movements of the mouse head and body were recorded for further analysis. Operators could adjust the software settings to change the spatial frequency and the relative contrast of the stimulus gratings.

Visual analyses were performed and modified according to methods described in recent studies (Kretschmer, Tariq, Chatila, Wu, & Badea, 2017; Prusky et al., 2004; Schmucker, Seeliger, Humphries, Biel, & Schaeffel, 2005; Tsai, Joachimsthaler, & Kremers, 2017; Umino et al., 2008). In the VA test, stimulus gratings were set at 100% contrast on the monitor with 7 ± 3 lx of the backlight illuminance (measured from the mouse position). Episodic stimuli consisted in full-screen square wave was displayed at spatial frequencies of 0.033, 0.055, 0.082, 0.164, 0.328, and 0.437 cycle per degree (cpd) with a constant rotational speed of 12 degrees per second drifting in two directions (0 and 180 degrees). The observer recorded the mouse head and body movements until they were no longer coordinated with the stimulus gratings. Thence, they determined the VA threshold level of the mouse optomotor response. In VCSF test, the VCSF threshold was determined using ten different contrast levels ranging from 10, 20, 30, 40, 50, 60, 70, 80, 90, 100% of the relative grating contrast at spatial frequency of 0.033, 0.055, 0.082, 0.164, 0.328 and 0.437 cpd, and with a constant rotational speed of 12 degrees per second drifting in two directions (0 and180 degrees), the illuminances were maintained in 7 ± 3 lx.

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