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Maqui berry (*Aristotelia chilensis*) and the constituent delphinidin glycoside inhibit photoreceptor cell death induced by visible light

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ABSTRACT

The protective effects of maqui berry (*Aristotelia chilensis*) extract (MBE) and its major anthocyanins [delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G)] against light-induced murine photoreceptor cells (661W) death were evaluated. Viability of 661W after light treatment for 24 h, assessed by the tetrazolium salt (WST-8) assay and Hoechst 33342 nuclear staining, was improved by addition of MBE, D3G5G, and D3S5G. Intracellular radical activation in 661W, evaluated using the reactive oxygen species (ROS)-sensitive probe 5-(and-6)-chloromethyl-2,7-dichlorodihydro fluorescein diacetate acetyl ester (CM-H_2DCFDA), was reduced by MBE and its anthocyanins. The anti-apoptosis mechanism of MBE was evaluated by light-induced phosphorylation of p38. MBE significantly suppressed the light-induced phosphorylation of p38. These findings indicate that MBE and its anthocyanidins suppress the light-induced photoreceptor cell death by inhibiting ROS production, suggesting that the inhibition of phosphorylated-p38 may be involved in the underlying mechanism.

1. Introduction

Everyday people are exposed to various visible light. It is generated by the sun as well as by a wide variety of artificial illumination sources such as fluorescent lights, light-emitting diodes (LED), and the monitors of computers, mobile phones, and televisions. However, excessive exposure to light can be a source of damage to the eye, as evidenced by photoreceptor degeneration in rats and mice following prolonged light exposure (LaVail, Gorrin, Repaci, Thomas, & Ginsberg, 1987; Noell, Walker, Kang, & Berman, 1996). In addition, sunlight exposure has a role in the progression of age-related macular degeneration (AMD) (Hirakawa et al., 2008) and can also contribute to retinitis pigmentosa (RP) as indicated in a human population-based study (Tomany, Cruickshanks, Klein, Klein, & Knudtson, 2004). In the USA, AMD and RP are the most frequent causes of blindness in adults (Dewan et al., 2006). Photoreceptor cell death is an irreversible injury and can cause night blindness and constriction of the visual field, leading to the loss of central vision. Light-induced photoreceptor cell death can be caused by a variety of cellular mechanisms that involve oxidative stress, reactive oxygen species (ROS), activation of caspase-1, and depletion of NF-κB (Krishnamoorthy et al., 1999). Light exposure also causes enhancement of the phosphorylation of p38 (stress activated protein kinase-2) of photoreceptor cells by ROS (Yang, Zhu, & Tso, 2007).

The human retina, in particular, requires large amounts of oxygen; thus, it readily generates ROS, such as superoxide anion radical ($\cdot O_2^-$) and hydrogen peroxide. Although the oxidising capability of these radicals is weak, they react with metals in living tissues and are immediately changed to hydroxyl radical (·OH) if exposed to ultraviolet light. This radical has great capacity to injure DNA and the cell membrane. The eye therefore depends on the presence of antioxidants such as ascorbic acid to protect the retina from light-induced free radical damage (Li, Tso, Wang, & Organisciak, 1985; Organisciak, Wang, Li, & Tso, 1985). However, oxidative stress conditions can overwhelm this internal antioxidant system, resulting in the progression of many diseases including retinal diseases.

Maqui berry [*Aristotelia chilensis* (Molina) Stuntz] is a plant of the Elaeocarpaceae family and cultivated in central and southern Chile. Maqui berry has a particularly high concentration of anthocyanins, and 8 anthocyanins found are glycosylated forms of delphinidin and cyanidin, such as delphinidin 3,5-*O*-diglucoside (D3G5G) and delphinidin 3-*O*-sambubioside-5-*O*-glucoside (D3S5G) (Fig. 1)

Abbreviations: BBE, bilberry extract; BCE, blackcurrant berry extract; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate acetyl ester; D3G5G, delphinidin 3,5-*O*-diglucoside; D3S5G, delphinidin 3-*O*-sambubioside-5-*O*-glucoside; MBE, maqui berry extract; PI, propidium iodide; ROS, reactive oxygen species; WST-8, 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H* tetrazolium monosodium salt.

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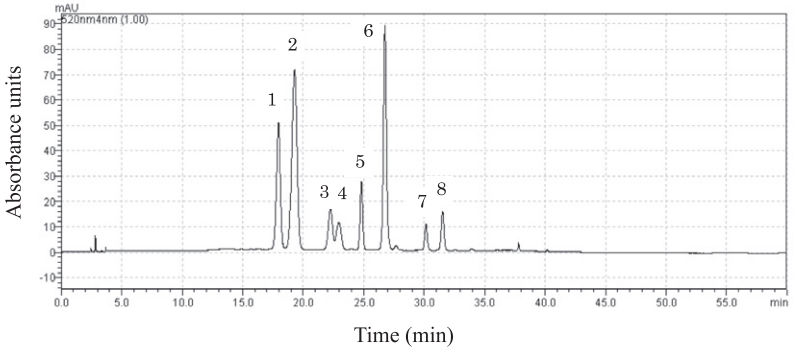
A



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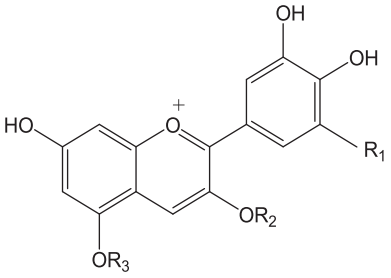

Fraction No.	Retention Time (min)	Anthocyanins	R_1	R_2	R_3
1	18.0	Delphinidin 3-*O*-sambubioside-5-*O*-glucoside	OH	Sam	Glu
2	19.3	Delphinidin 3,5-*O*-diglucoside	OH	Glu	Glu
3	22.3	Cyanidin 3-*O*-sambubioside-5-*O*-glucoside	H	Sam	Glu
4	23.0	Cyanidin 3,5-*O*-diglucoside	H	Glu	Glu
5	24.8	Delphinidin 3-*O*-sambubioside	OH	Sam	H
6	26.8	Delphinidin 3-*O*-glucoside	OH	Glu	H
7	30.2	Cyanidin 3-*O*-sambubioside	H	Sam	H
8	31.5	Cyanidin 3-*O*-glucoside	H	Glu	H

Fig. 1. HPLC separation of MBE. (A) Use of 520 nm as a selective wavelength allowed identification of 8 anthocyanins. The various peak numbers correspond to the 'Fraction No.' in (B). (B) Chemical structure of MBE. Glu, glucose; Sam, sambubiose.

(Maria, Cristina, Orlando, Julian, & Celestino, 2006). D3G5G and D3S5G are not contained in bilberry (*Vaccinium myrtillus* L.) or blackcurrant berry (*Ribes nigrum* L.) (Matsumoto, Nakamura, Iida, Ito, & Ohguro, 2006; Matsunaga et al., 2009). Many studies conducted on the biological activities of maqui berry extract (MBE) have reported, such as antioxidant (Miranda-Rottmann et al., 2002; Ruiz et al., 2010), antimicrobial (Mølgaard et al., 2011), cardioprotective (Cespedes, El-Hafidi, Pavon, & Alarcon, 2008), antidiabetic (Rojo et al., 2012), anti-inflammatory (Schreckinger, Wang, Yousef, Lila, & de Mejia, 2010), and α-glucosidase/α-amylase inhibitory (Rubilar et al., 2011) effects. Moreover, it has been previously shown that the protective effect of purple rice (*Oryza sativa* L.) bran extract and cyanidin of the main constituents against light induced retinal damage (Tanaka et al., 2011). However, the effects of MBE, D3G5G, and D3S5G on light-induced photoreceptor cell death have not yet been extensively examined.

The purpose of the present study was to examine the potential protective effects of MBE and its constituents (D3G5G and D3S5G) against murine photoreceptor cells (661W) death induced by light exposure. Furthermore, MBE was compared with data obtained for other berry fruits of bilberry and blackcurrant berry. The mechanisms underlying the effects of MBE on ROS production and induction of phosphorylation of p38 by light were also investigated.

2. Materials and methods

2.1. Materials

Maqui berry (*A. chilensis*) extract (MBE), blackcurrant berry (*R. nigrum* L.) extract (BCE), and bilberry (*V. myrtillus* L.) extract (BBE) were supplied by Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan), which were extracted with aqueous ethanol. Delphinidin 3,5-*O*-diglucoside and delphinidin 3-*O*-sambubioside-5-*O*-glucoside were purchased from Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). Delphinidin, cyanidin, malvidin, and peonidin were purchased from Extrasynthese (Genay Cedex, France). A Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Hoechst 33342 and propidium iodide (PI)