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Protective effect of fucoxanthin isolated from *Ishige okamurae* against high-glucose induced oxidative stress in human umbilical vein endothelial cells and zebrafish model

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ABSTRACT

High glucose induced oxidative stress is implicated in intracellular toxicity in tissue and blood vessel such as oxidative stress, lipid peroxidation, and cell death. In this study, we attempted to investigate protective effects of fucoxanthin isolated from *Ishige okamurae* against high glucose induced oxidative stress in human umbilical vein endothelial cells (HUVEC) and zebrafish model. The ROS generation, lipid peroxidation and cell death were significantly reduced in both the fucoxanthin treated HUVEC and zebrafish *in vivo* model, compared to those of both negative controls. This study indicates that fucoxanthin could protect cells and organ injury against oxidative stress induced by high glucose *in vitro* HUVEC and *in vivo* zebrafish model.

1. Introduction

The chronic diseases have attracted much attention in recent years, especially for diabetes, obesity, hypertension and hyperlipidemia (Vizoris & Tarasuk, 2003). Among them, diabetes is one of the most serious metabolic diseases that have been known to be caused by metabolic disorders (Niazi & Kalra, 2012). Diabetes is characterized by an increase in the blood glucose level by insulin resistance (Soumya & Srilatha, 2011). Recently, many researchers have reported that the high glucose level in blood induces a wide range of disorders such as heart

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disease, kidney damage and stroke (Kawahito, Kitahata, & Oshita, 2009). Moreover, high glucose level in blood can cause oxidative damage to vessel, brain and tissue, leading to disease (Ceolotto et al., 2007). Previously reported studies have shown that antioxidants could protect oxidative damage induced by high glucose *in vitro* and *in vivo* (Tsubouchi et al., 2005).

Moreover, several studies have reported that antioxidative effects improve dysfunction of the metabolisms and oxidative damage by high glucose level in blood (Sheu et al., 2008). Recently, many researchers have reported that *radix hedysari*, *Cordyceps militaris* and *olea europaea* L. have the protective effects against oxidative damage caused by the treatment of high glucose *in vitro* and *in vivo* (Chu, Chien, & Duh, 2011; Kaeidi et al., 2011; Liu et al., 2012a). The main components of brown algae include polyphenols, pigments, polysaccharides, minerals and amino acids (Kang et al., 2012a, 2012b). In previous studies, pigments of brown algae have been reported to exhibit various biological activities such as antioxidant, anti-inflammation and anti-tumor effect (Budhiyanti, Raharjo, Marseno, & Lelana, 2012). The extract of *Ishige okamurae* has shown various biological activities *in vitro* and *in vivo* such as antioxidant, anti-diabetes and neuro-protective effect (Heo et al., 2012; Min, Kim, Jeon, & Han, 2011). Among these pigment, fucoxanthin are found in brown algae and chrysophyta, have been reported to improve oxidative stress and metabolic syndrome such as anti-diabetes and anti-obesity (Kang et al., 2011; Kumar, Hosokawa, & Miyashita, 2013; Liu, Liang, & Hu, 2011; Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2005; Maeda, Hosokawa, Sashima, & Miyashita, 2007; Nishikawa, Hosokawa, & Miyashita, 2012; Woo et al., 2010). Therefore, in the present study, we investigated the protective effects of fucoxanthin against high glucose level induced oxidative damage in human umbilical vein endothelial cells (HUVEC) and zebrafish model.

2. Materials and methods

2.1. Extraction and isolation of fucoxanthin

The marine brown alga, *Ishige okamurae*, was collected along the coast of Jeju Island, Korea, between October 2011 and March 2012. The sample was washed three times with tap water to remove salt, epiphytes, and sand attached to the surface. They were then carefully rinsed with fresh water, and maintained in a medical refrigerator at –20 °C. Thereafter, the frozen samples were lyophilized and homogenized using a grinder prior to extraction. The algal powder was extracted three times with 80% aqueous methanol, and was evaporated under vacuum at 40 °C. The methanol extract was partitioned with chloroform, and the chloroform extract was fractionated via silica column chromatography with the stepwise elution of a chloroform–methanol mixture (100:1–1:1) to generate the separated active fractions. The combined active fraction was then applied to a Sephadex LH-20 column (GE Healthcare, Uppsala, Sweden) saturated with 100% methanol, and finally purified via reversed-phase HPLC (Thermo Fisher Scientific, San Jose, CA, USA) using a Waters HPLC system equipped with a Waters 996 photodiode array detector and a C^{18} column (150 × 20 mm, 4 μm, J'sphere ODS-H80; YMC Co., Kyoto, Japan) via stepwise elution with a methanol–water gradient (UV range: 440 nm, flow rate: 0.8 ml/min). The purified compounds were definitively identified via comparisons of their LC/MS, ^{1}H, and ^{13}C NMR data with those in the relevant literature (Heo et al., 2008). The purity of fucoxanthin (Fig. 1) was >95%, based on the peak area of all components absorbed at each specific wavelength in HPLC analysis. Fucoxanthin was dissolved in dimethyl sulphoxide and employed in experiments in which the final concentration of dimethyl sulphoxide in culture medium was adjusted to <0.01%.

2.2. Cell culture

HUVEC cultures were maintained at 37 °C in an humidified atmosphere containing 5% CO_2, in an endothelial cell growth medium-2 EBM-2 supplemented with ascorbic acid, 2% fetal bovine serum (FBS), hydrocortisone, human fibroblast growth factor (hFGF), long R insulin-like growth factor-1 (R3-IGF-1), gentamicin sulphate (CA-1000, ph of 7) and heparin as described by the manufacturer (Clonetics, Walkersville, MD, USA).

2.3. Assay of intracellular ROS levels in HUVEC cells

Intracellular ROS levels were measured by the dichlorofluorescein assay (Wang & Joseph, 1999). 2′,7′-Dichlorodihydrofluorescein diacetate (DCF-DA) can be deacetylated in cells, where it can react quantitatively with intracellular radicals to be converted into its fluorescent product, DCF, which is retained within the cells. Therefore, DCF-DA was used to evaluate the generation of ROS in oxidative stress. Cells (4×10^4 cells/well) were seeded to 24-well plates and preincubated with glucose (5.5 mM or 30 mM) for 48 h, and then incubated without or with the indicated concentrations of fucoxanthin for 20 h, after that the cells were washed with phosphate buffered saline (PBS) and incubated with 5 μM DCF-DA for 30 min at room temperature. Fluorescence was measured using a fluorescence plate reader.

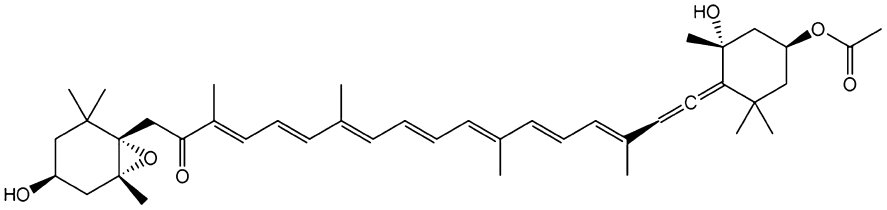

Fig. 1 – Chemical structure of fucoxanthin from *Ishige okamurae*.