Prevention of Ultraviolet B-induced Lens Oxidative Damage in Mice by *Dunaliella salina*, A Carotenoids-Rich Alga

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Abstract: The present study examined the protective effects of *Dunaliella salina* (*D. salina*) on ultraviolet B (UVB) radiation-induced lens oxidative damage in male ICR mice. Lens oxidative damage was induced by exposure UVB radiation. Animal were orally administered (gavage) *D. salina* at doses of 0, 123 and 615 mg/kg body weight/day for eight days. Lens glutathione (GSH) and malondialdehyde (MDA) levels as well as the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in lens were measured to monitor lens injury. The results showed that UVB irradiation caused significant damages to lens, including decreased the activities of SOD, catalase and GSH-Px, and GSH content in lens whereas increased lens MDA content, compared with control group. Treatment with *D. salina* could significantly ($p < 0.05$) ameliorate lens oxidative damages, as evidenced by increased the activities of SOD, catalase and GSH-Px, and GSH content and decreased the MDA content in lens when compared with UVB-treated group. Those results demonstrate that *D. salina* exhibits potent protective effects on UVB radiation-induced lens oxidative damage in mice, likely due to both the increase of antioxidant enzymes activities and the inhibition of lipid peroxidation.
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1. Introduction

Ultraviolet (UV) irradiation is the most frequent cause of radiation injury to the eye, which is affected by oxidative stress due to its physical and metabolic characteristics. Particularly, the lens is most affected by oxidative damage caused by UVB radiation, because it is an avascular structure and has a constant and spare production of lenticular proteins [1]. Many studies reported that antioxidants can effectively prevent and cure UVB-induced protein oxidation and photoperoxidation of lipids in lens [2-3]. A major defense mechanism for prevention and treatment of lens oxidative damage comprises reducing the production of reactive metabolites by raising the levels of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), and decreasing lipid peroxidation [1, 4].

Dunaliella salina (*D. salina*) is a type of unicellular biflagellate green alga from Chlorophyceae gender without a rigid cell wall structure and can yield three major valuable products, glycerol, β-carotene and proteins [5-8] *D. salina* is safe and good for human health, because *D. salina* contains abundant β-carotene and other carotenoids including lutein, zeaxanthin and α-carotene [9-10]. Recently, our group demonstrated that *D. salina*, which contains abundant carotenoids and xanthophylls, is efficient antioxidants against a variety of oxidative stress *in vitro* and *in vivo* [8, 10]. It is well know that the major precursor of vitamin A is β-carotene, which quenches excited sensitizer molecules and singlet oxygen. Additionally, lutein and zeaxanthin are found in high concentrations in some ocular tissues, such as the macula, retina and lens [11-12]. Several studies have demonstrated that high dietary xanthophylls intake is associated with reduced cataract prevalence [13-14].

In consideration of excellent antioxidant activities of *D. salina*, we hypothesized that administration of *D. salina* may enhance the antioxidant defense system and thus provide against UVB-induced lens oxidative damage in mice. In the present study, male ICR mice were orally treated with *D. salina* daily accompanied by UVB exposure for a period of 8 days. Lens GSH and MDA levels, as well as SOD, catalase and GSH-Px activities in lens tissues, were also measured to monitor lens injury.

2. Material and Methods

D. salina material

Commercially available spray-dried preparations of *D. salina* paranoids cultured in the outdoor cultivation pool at GONG BIH Enterprise Co., Ltd (Yunlin City, Taiwan) were suspended in distilled water prior to use. The quality of *D. salina* powder was described and provided by the company. The carotenoid contents in the *D. salina* were measured as described previously [10].

Animals

Male ICR mice (20 ± 2 g) were obtained from the Animal Department of BioLASCO Taiwan Company and were allowed to quarantine and acclimate for a week prior to experimentation. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature and humidity controlled room. Food and water were available *ad libitum*. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline. All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Treatment

The animals were randomly divided into four groups each consisting of ten mice. Group I served as the normal control and was given olive oil by gavage daily for a period of 8 days. For inducing lens damage in mice, eyes of animals in Groups II, III and IV were exposed to UVB irradiation by the method of Tanito and colleagues [18] with slight modification. After anaesthesia was induced by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight), both of their eyes were exposed to 560 $\mu W/cm^2$ of UVB light (UVLS-26; UVP Inc., Cambridge, UK) for 180 seconds in a darkroom. During irradiation, the mice were confined in an adjustable retaining cage that protected most of the animal, except the head from the UV light. The wavelength of the light source peaked at 312 nm (range, 280-315 nm). The energy output was measured with a UV detector (USB4000) with a sensor (CC-3-UV-S; both from Ocean Optics, Inc., FL, USA). The entire UVB irradiation course was completed in a consecutive 5-day period. After UVB light exposure, Group II served as the UVB control and was orally administered olive oil daily for a period of 8 days. Groups III and IV were orally administered the *D. salina* dissolved in olive oil at doses of 123 and 615 mg/kg, respectively, daily for a period of 8 days. At the end of the experiment, all animals were sacrificed by CO_2 for euthanasia. Eye samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible, and immediately stored at -70ºC until further analysis.

Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation was done by measuring the concentration of TBARS in liver using the method of Berton and colleagues [15]. The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. In brief, samples were mixed with TBA reagent consisting of 0.375% TBA and 15% trichloroacetic acid in 0.25N hydrochloric acid. The reaction mixtures were placed in a boiling water bath for 30 min and centrifuged at 1,811g for 5 min. The supernatant was collected and its absorbance was measured at 535 nm. The results were expressed as nmole/mg protein using the molar extinction coefficient of the chromophore ($1.56 \times 10^{-5}\ M^{-1}cm^{-1}$).

Measurement of SOD, catalase, GSH-Px and GSH in lens

Lens homogenates were prepared in cold Tris-HCl (5 mmol/L, containing 2 mmol/L EDTA, pH 7.4) using a homogenizer. The unbroken cells and cell debris were removed by centrifugation at 10,000 rpm for 10 min at 4ºC. The supernatant was used immedi-ately for the assays for SOD, catalase, GSH-Px and GSH. The activities of all of these enzymes and GSH levels were determined following the instructions in the Randox Laboratories Ltd kit (Antrim, United Kingdom).

Statistical analysis

All values are expressed as the means ± SDs. Comparisons between any two groups were performed using one-way analysis of variance (ANOVA), followed by the Munnett multiple comparison test. The calculations were performed with SPSS software (Drmarketing Co., Ltd. New Taipei City, Taiwan). Statistically significant differences between groups were defined as $p < 0.05$.

3. Results

3.1 Effect of *D. salina* on lipid peroxidation after UVB exposure in the lens

MDA level is widely used as a marker of free radical mediated lipid peroxidation injury. The results of the MDA assays in the lens are shown in Fig. 1. MDA levels in the UVB-treated group (1.62 ± 0.25 nmol/mg protein) were significantly higher than that in the control group (0.98 ± 0.10 nmol/mg protein, $p < 0.05$). However, MDA levels in the *D. salina* treated group (1.21 ± 0.11 and 1.02 ± 0.14 nmol/mg protein at dose of 123 and 615 mg/kg, respectively) were significantly lower than that in the UVB-treated

group ($p < 0.05$). These findings indicated that the free radicals being released in the lens were effectively scavenged when treated with *D. salina.*

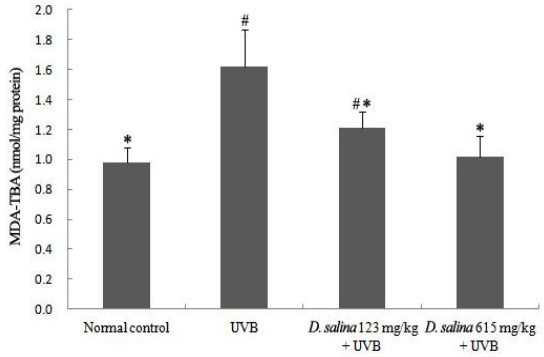

Fig. 1. Effects of *D. salina* on lens MDA-TBA level in UVB-induced lens oxidative damage in mice. Data are mean ± SD of all the animals in a group; # $p < 0.05$ compare with normal control; * $p < 0.05$ compare with UVB-treated group.

3.2 Effect of *D. salina* on GSH level after UVB exposure in the lens

GSH, a nonenzymatic antioxidant in the detoxification pathway, is abundant in the lens that reduces H_2O_2, hydroperoxides (ROOH) and photo-oxidation. The effects of *D. salina* on GSH levels are summarized in Fig. 2. UVB irradiation caused a significantly decrease on the GSH levels in the lens (4.25 ± 0.74 nmol/mg protein) as compared to the normal control group (11.49 ± 1.10 nmol/mg protein). In contrast with UVB-treated group, mice treated with *D. salina* at dose of 123 and 615 mg/kg showed a significantly increase the levels of GSH upto 170% (Fig. 2). These results suggested that *D. salina* provides protection against UVB-induced GSH depletion in the lens.

3.3 Effect of *D. salina* on antioxidant enzyme activities after UVB exposure in the lens

To further elucidate the reduction of MDA accumulation and GSH depletion in the UVB-exposed lens, we examined the status of antioxidant enzymes, SOD (Fig. 3A), catalase (Fig. 3B) and GSH-Px (Fig. 3C), in the lens. The activities of lens SOD, catalase and GSH-Px in the UVB-treated group were significantly decreased by 36%, 33% and 54%, respectively, when compared with the normal control group. In contrast, there was a significant increase ($p < 0.05$) of SOD, catalase and GSH-Px activity in the *D. salina*-treated groups at a dose of 123 and 615 mg/kg, respectively, as compared to the UVB treated group. These findings indicated that administration of *D. salina* effectively restored/maintained the activities of lens SOD, catalase and GSH-Px, that was decreased by UVB exposure in the lens.

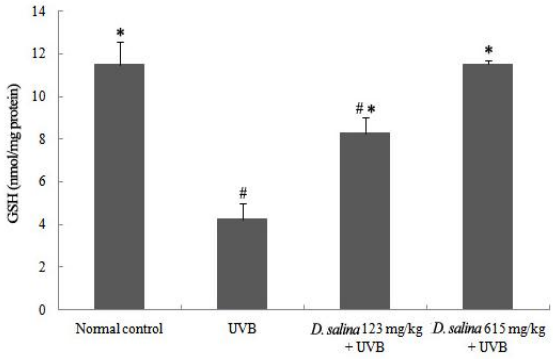

Fig. 2. Effects of *D. salina* on lens GSH amount in UVB-induced lens oxidative damage in mice. Data are mean ± SD of all the animals in a group; # $p < 0.05$ compare with normal control; * $p < 0.05$ compare with UVB-treated alone group.

4. Discussion

Previous study, we observed that *D. salina*, which contains abundant carotenoids and xanthophylls, is efficient antioxidants against a variety of oxidative stress [10]. Various epidemiological studies have indicated that dietary lutein and zeaxanthin play a crucial role in the prevention of the oxidation of lens proteins [16-18]. Moreover, lutein and zeaxanthin were also identified in the human lens [10-11]. Therefore, we considered that *D. salina* may be useful in the prevention of various damages induced by oxidative stress. In the present study, the capability of *D. salina* to protect against UVB radiation-induced oxidative damage in the lens was investigated.

Earlier studies have demonstrated the harmful effects of UVB radiation from sunlight in the lens [19-20]. UVB-induced reactive oxygen species, such as hydrogen peroxide, singlet oxygen, superoxide anions and hydroxyl radicals, are reported to initiate peroxidation [21] and react to proteins or lipids, leading to membrane lipid peroxidation and finally cell necrosis [20]. The main ROS generation occurs in the lens due to high exposure to UVB radiation. Lipid peroxidation by the generation of ROS is one of the principal mechanisms of UVB radiation-induced lens injury [1]. Moreover, the initiation of oxidative stress related to various tissue injuries, cell death and the progression of many acute and chronic diseases is generally believed to be induced by increased lipid peroxidation [22-23]. The capacity of an antioxidant to scavenge reactive oxygen species has been recognized as an important factor that contributes to protective effects in the lens. Indeed, considerable experimental animal models have reported that several antioxidant agents, such as ascorbic acid [24-25], vitamin E [2], α-tocopherol [26] and *N*-acetylcysteine [27], reduced UVB

radiation-induced phototoxicity effects by prevention of lipid peroxidation. In the present study, UVB radiation-induced oxidative damage caused a significantly increase in MDA levels in the lens. Treatment with *D. salina* significantly reversed these changes. The administration of *D. salina* caused a significant decrease in MDA levels compared to the UVB radiation-induced oxidative damage group.

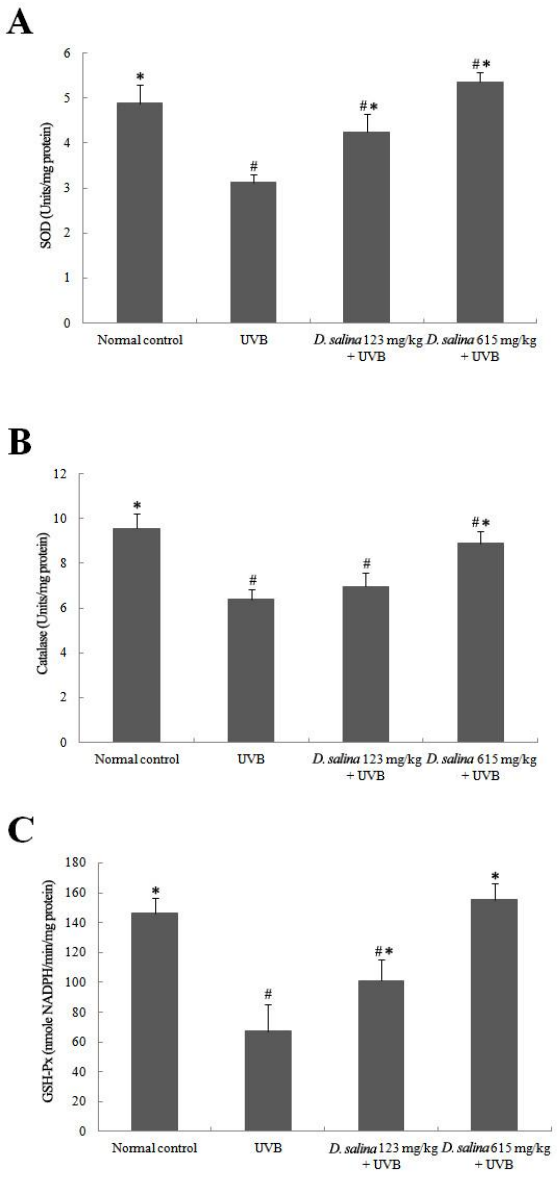

Fig. 3. Effects of *D. salina* on lens antioxidant enzymes (A) SOD, (B) catalase and (C) GSH-Px activities in UVB-induced lens oxidative damage in mice. Data are mean ± SD of all the animals in a group; $^{\#}p < 0.05$ compare with normal control; $^{*}p < 0.05$ compare with UVB-treated alone group.

Previous studies on the mechanism of UVB radiation-induced lens damage showed that GSH is an extremely efficient intracellular antioxidant for oxidative stress and it reduces H_2O_2, hydroperoxides (ROOH) and photooxidation cause by UVB radiation exposure [1]. GSH is easily oxidized to GSSG by xenobiotic compounds, and there may additionally be reaction with any of the selenium-containing GSH-Px isozymes that may subsequently result in the reduction of GSH levels. GSSG is either rapidly reduced by glutathione reductase and NADPH or utilized in the protein-folding process in the endoplasmic reticulum [28]. Because of these recycling mechanisms, GSH is major defense mechanism against photo-oxidation in the lens [29]. The oxidation-reduction cycle of this tripeptide links dietary antioxidants such as ascorbate, riboflavin, carotenoids and tocopherols with the prevention of photo-oxidation [4]. The level of GSH in the lenticular epithelium is as high as that in the liver and its concentration decreases by exposure to UV radiation [30]. In the present study, the content of GSH in the lens was significantly decreased in UVB radiation-exposed mice compared with control mice. Conversely administration of *D. salina* to UVB radiation-exposed mice significantly elevated GSH content in the lens compared to the untreated group, indicating that *D. salina* can protect against the UVB radiation-induced GSH depletion in the lens.

The intracellular antioxidant status is always maintained at equilibrium in mammalian cells. Dietary supplementation with extra natural antioxidants mainly help the intracellular antioxidant defense system that includes non-enzymatic antioxidants (e.g., GSH, bilirubin, and vitamins E and C) and enzymatic antioxidants such as SOD, catalase and GSH-Px in protecting cells and organs against ROS-induced oxidative damage [31]. Moreover, oxidative stress associated with increased ROS is known to hasten cataract formation and lens oxidetive damage in laboratory rodent models [32]. Therefore, the enzymatic antioxidant activities and/or the inhibition of free-radical generation are important in terms of protecting the lens from UVB-induced oxidative damage [1, 26].

Each of these enzymes catalyzes the reduction of a particular type of ROS. Superoxide anions are catalyzed in most tissues of the body, including the lens, into hydrogen peroxide (H_2O_2) by SOD, which is an exceedingly effective defense enzyme. H_2O_2 can become highly toxic because it produces the hydroxyl radical. This toxicity is prevented by catalase and GSH-Px. Catalase is a haemeprotein in all aerobic cells that metabolize H_2O_2 to oxygen and water. GSH-Px is a selenoprotein that catalyses the reduction of H_2O_2 and hydroperoxides to non-toxic

products [1, 33]. These antioxidant enzymes protect the lens against UVB radiation-induced lipid peroxides or free radicals. Therefore, these anti-oxidant enzymes are easily deprived of their activity by lipid peroxides or free radicals, resulting in their decreased activities in UVB radiation exposure [4, 34]. In the present study, SOD, catalase and GSH-Px levels were significantly elevated by administration of *D. salina* to UVB radiation-damaged lens, suggesting that it has the ability to restore and/or maintain these enzymes' activities in UVB radiation-induced oxidative damaged in the lens.

5. Conclusion

The results of this study demonstrate that *D. salina* was effective for the prevention of UVB radiation-induced oxidative stress in the lens. Our results show that the protective effects of *D. salina* may be due to both an increase in the activity of the antioxidant defense system and an inhibition of lipid peroxidation. The inhibitory effects of a dietary *D. salina* may be useful as a protective agent against UVB radiation-induced lens damage *in vivo*.

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