Antioxidant potential of meso-zeaxanthin a semi synthetic carotenoid

Alikkunjhi P. Firdous, Korengath C. Preethi, Ramadasan Kuttan *

Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala 680 555, India

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ABSTRACT

Semi synthetic carotenoid meso-zeaxanthin was evaluated for its antioxidant potential in vitro and in vivo. Meso-zeaxanthin was found to scavenge superoxide radicals, hydroxyl radicals and inhibited in vitro lipid peroxidation. Concentrations needed for 50% inhibition (IC50) were 27.0, 3.5 and 3.2 μg/ml, respectively. It scavenged 2,2-azobis-3-ethylbenzthiozoline-6-sulphonic acid and 2,2-diphenyl-1-picryl hydrazyl radicals and IC50 were 46.5, 6.25 μg/ml, respectively. It also scavenged nitric oxide radicals and IC50 was found to be 2.2 μg/ml. Oral administration of meso-zeaxanthin inhibited superoxide radicals generated in macrophages by 25.2%, 50.1% and 67.2% at doses of 50, 100 and 250 mg/kg b.wt., respectively. One month oral administration of meso-zeaxanthin to mice significantly increased catalase, superoxide dismutase, glutathione and glutathione reductase levels in blood and liver. Levels of glutathione peroxidase and glutathione-S-transferase were also found to be increased in the liver, in a dose dependent manner. These results showed that meso-zeaxanthin has significant antioxidant activity in vitro and in vivo.

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1. Introduction

Free radical production is ubiquitous in all respiring organisms, and is enhanced in many disease states. Most free radicals in biological systems are derivatives of oxygen (reactive oxygen species, “ROS”) but there are also derivatives of nitrogen (reactive nitrogen species, “RNS”). Oxidative and nitrosative stress occurs when the rate of production of ROS and RNS exceed the antioxidant capability of the cell. In such cases, ROS/RNS interact with and modify the cellular proteins, lipids and DNA, which results in altered cell function (Singh & Agarwal, 1995). Although free radicals are formidable weapons in the arsenal of our immune system, they have been implicated in the etiology of various diseases including inflammation, cancer, Alzheimer’s disease, ischaemic reperfusion injury and a myriad of other disease conditions. The link between free radicals and disease processes led to considerable research to develop nontoxic drugs that can scavenge the free radicals. Several plant extracts and products have been shown to possess significant antioxidant potential (Sabu & Kuttan, 2003).

Carotenoids are red, yellow and orange coloured natural fat soluble pigments found in plants and have been reported to have a number of biological actions, like antioxidant activity, immuno enhancement, inhibition of mutagenesis and transformation, and regression of premalignant lesions (Krniski, 1993). Zeaxanthin [(3R,3’R)-β,β-carotene-3,3’-diol] and lutein [(3R,3’R,6’R)-β-c-carotene-3,3’,6-triols] are the non-provitamin A carotenoids, commonly found in green and yellow vegetables. These xanthophyll carotenoids are the predominant carotenoids found in human retina and their concentration is greatest in the fovea centralis of the macula lutea, where they constitute the macular pigment and serve to decrease the risk of age-related macular degeneration (Beatty, Boulton, Henson, Koh, & Murray, 1999) and to protect the retinal pigment epithelium against a photo oxidative damage initiated in part by light absorption (Broekmans et al., 2002; Snodderly, 1995). There is good evidence that this protection is mainly due to their antioxidant properties (Zhang, Cooney, & Bertran, 1991).

Recently a third carotenoid, meso-zeaxanthin [(3R,3’S)-β,β-carotene-3,3’-diol] was found to be effective in the ageing macula to maintain its structural density (Bone, Landrum, Alvarez-Correa, Etienne, & Ruitz, 2003). Meso-zeaxanthin resides directly over the centre of the macula, where light is focused and where the strongest need for hazardous actinic blue light protection exists (Landrum, Bone, Moore, & Gomez, 1999). It is startling that the retina accumulates only the xanthophylls meso-zeaxanthin, zeaxanthin and lutein, whilst not even traces of other carotenoids are found in this tissue (Landrum & Bone, 2001) (Fig. 1).

Unlike lutein and zeaxanthin, meso-zeaxanthin is not found in the diet, and is undetectable in the blood serum. But this carotenoid is of such importance to the eye that it is exclusively synthesised there from ingested lutein. If taken as a supplement, meso-zeaxanthin is absorbed into the blood stream and effectively increases macular pigment levels (Bone et al., 2003). In the present work, we have investigated the antioxidant potential of...
Zeaxanthin and meso-zeaxanthin were supplied by Omni Active Health Technologies Pvt. Ltd., Mumbai. In order to get a uniform suspension of zeaxanthin for in vitro studies, zeaxanthin and meso-zeaxanthin powders were dissolved in hexane (10 mg/10 ml) and 10 μl of triton X 100 was added and further evaporated to dryness and made up the volume to 10 ml with distilled water. For in vivo studies, a 5% suspension was prepared in sunflower oil.

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2.4.6. Determination of nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent (Green et al., 1982). Stock solution (10 mM) of sodium nitroprusside was prepared in PBS (pH 7.4). Various concentrations of carotenoids (0.1–4 μg) and sodium nitroprusside (1 mM) in PBS in a final volume of 3 ml were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the solution was removed and diluted with 0.5 ml of Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during the diazotisation of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm. The percentage inhibition of nitric oxide was calculated by comparing with the control.

2.4.7. Ferric reducing antioxidant power assay (FRAP)

The Ferric reducing ability was measured at low pH (Benzie & Strain, 1996). The FRAP reagent contained 2.5 ml 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) solution, 2.5 ml ferric chloride solution (20 mM) and 25 ml acetate buffer. Freshly prepared FRAP reagent (900 μl) was mixed with various concentrations of sample (10–100 μg) and incubated at 37 °C for 15 min. Read absorbance at 595 nm against distilled water. Values are expressed as milli moles of ferrous chloride formed.

2.5. Determination of the effect of meso-zeaxanthin on PMA-induced superoxide radical generation in peritoneal macrophages

Male Swiss albino mice (4–6 weeks) weighing 20–25 g were used for the study. Animals were divided into four groups (three animals/group). All the animals were injected (i.p) with sodium caseinate (5%) to elicit macrophages. Group I was kept as control. Group II, III and IV were treated with single dose of meso-zeaxanthin (50, 100 and 250 mg/kg b.wt., respectively). On the fifth day after 1 h of drug administration, peritoneal macrophages elicited by sodium caseinate were activated in vivo after 1 h of drug administration, peritoneal macrophages elicited thin (50, 100 and 250 mg/kg b.wt., respectively). On the fifth day Group II, III and IV were treated with single dose of meso-zeaxanthin (100 mg/animal). After 3 h, peritoneal macrophages were harvested. The effect of meso-zeaxanthin on the inhibition of superoxide generation in the macrophages was measured by inhibition in the reduction of NBT to formazan by the method of Dwivedi, Verna, and Ray (1992). The percentage inhibition was determined by comparing the absorbance values of untreated and treated animals.

2.6. Determination of in vivo antioxidant activity of meso-zeaxanthin

Twenty-four Swiss albino male mice were divided into 4 groups of 6 animals and they were treated orally with meso-zeaxanthin dissolved in sunflower oil at different doses for 30 days.

- Group I: Normal.
- Group II: Control treated with Sunflower oil (0.2 ml).
- Group III: meso-zeaxanthin – 100 mg/kg b.wt.
- Group IV: meso-zeaxanthin – 250 mg/kg b.wt.

At the end of the experiment, animals were sacrificed, and blood was collected by heart puncture and liver was excised and washed in ice-cold Tris–HCl buffer (0.1 M, pH 7.4), and cytosolic samples of liver homogenate were prepared by centrifugation at 10,000 rpm for 30 min at 4 °C.

Estimation of the total protein was carried out by the method of Lowry, Rosenbrough, Farr, and Randall (1951). Hemoglobin was estimated by the cyanmethemoglobin solution using Drabkin's method (Drabkin & Austin, 1932). The following parameters were assayed in both blood and liver to assess the oxidative stress.

Superoxide dismutase (SOD) activity was measured by the NBT reduction method of Mc Cord and Fridovich (1969). Catalase activity was estimated by the method of Aebi (1974) by measuring the rate of decomposition of hydrogen peroxide at 240 nm. Glutathione (GSH; γ-glutamyl cysteine glycine) activity was assayed by the method of Moron, Deipiere, and Manner Vick (1979), based on the reaction with DTNB. The assay of glutathione peroxidase (GPX) was carried out by the method of Hafeman, Sundae, and Houestra (1974) based on the degradation of H2O2 in the presence of GSH. Glutathione reductase (GR) activity was measured by the method of Racker (1955), where the amount of reduced form of NADP consumed during the conversion of GSSG to GSH was estimated. The method of Habig, Pabst, and Jakoby (1974) was followed to assay the activity of glutathione-S-transferase (GST) based on the rate of increase in conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB).

2.7. Statistical analysis

The values were expressed as mean ± standard deviation (SD). Statistical evaluation of the data was done by one way ANOVA followed by Dunnet’s test (post-hoc) using Instat 3 software package.

3. Results and discussion

3.1. In vitro antioxidant activities of zeaxanthin and meso-zeaxanthin

Both zeaxanthin and meso-zeaxanthin were found to scavenge superoxide, hydroxyl radicals and inhibited tissue lipid peroxidation in vitro in a concentration dependent manner (Fig. 2). The concentrations of zeaxanthin and meso-zeaxanthin needed for scavenging (IC50) of superoxide generated by photo reduction of riboflavin were found to be different and meso-zeaxanthin was found to be superior to zeaxanthin as concentration needed for 50% inhibition was half that of zeaxanthin. However it was found that zeaxanthin was found to be a better scavenger for hydroxyl radical generated by Fe2+/ascorbate/EDTA/H2O2 system (Fenton reaction). Similarly zeaxanthin was found to be better inhibitor of lipid peroxidation compared to that of meso-zeaxanthin. Stable free radicals such as DPPH and ABTS were effectively scavenged by zeaxanthin and meso-zeaxanthin. In the case of DPPH, meso-zeaxanthin was found to be superior to zeaxanthin however, ABTS radical was found to be scavenged much better with zeaxanthin. Nitric oxide, another free radical in biological system, which is produced during oxidative stress has a major role in disease causation, was also found to be scavenged by both these carotenoids. The IC50 values for zeaxanthin was again found to be lower than meso-zeaxanthin. The effect of meso-zeaxanthin on zeaxanthin and meso-zeaxanthin were found to be almost similar, 0.2 and 0.23 mM, respectively. In summary the free radical scavenging activities of meso-zeaxanthin was found to be very much similar to that of zeaxanthin even though there are some minor variations. However the IC50 values in both cases were significantly low compared to other known antioxidants (Table 1).

3.2. Effect of meso-zeaxanthin on PMA-induced superoxide radical generation

The effect of meso-zeaxanthin on in vivo superoxide scavenging was determined by PMA-induced superoxide generation method. Superoxide radical generated during the activation with PMA in sodium caseinate-induced macrophages was found to be scavenged after oral administration of meso-zeaxanthin in a concentration dependent manner. The percentage inhibition was 25.2%, 50.1% and 67.2% for 50, 100 and 250 mg/kg b.wt., respectively.
3.3. Effect of meso-zeaxanthin administration on antioxidant enzymes and glutathione

Effect of meso-zeaxanthin on the antioxidant enzymes in the blood and serum of mice after given for a period of thirty days is shown in Table 2. Catalase was found to be significantly increased in animals treated with meso-zeaxanthin (p < 0.001). SOD was also found to be significantly elevated in 250 mg/kg b.wt. group (p < 0.001). GR in the serum was found to be significantly increased in 100 mg/kg b.wt. (p < 0.01) and 250 mg/kg b.wt. (p < 0.001)
The human body has several mechanisms to counteract damage by free radicals and other reactive oxygen species. These act on different oxidants as well as in different cellular compartments. One important line of defence is a system of antioxidant enzymes including SOD, catalase, GPX and GR. SOD is a metalloprotein, converts two superoxide radicals into hydrogen peroxide and O₂. One important line of defence is a system of antioxidant enzymes including SOD, catalase, GPX and GR. SOD is a metalloprotein, converts two superoxide radicals into hydrogen peroxide and O₂. These radicals are generated inside the body during the normal metabolism or in presence of xenobiotics. The stable free radicals DPPH and ABTS generated after the administration of phorbol esters in the liver of meso-zeaxanthin treated groups. All these results show that carotenoid meso-zeaxanthin has a profound effect on the antioxidant defence system both in vitro and in vivo. In addition, meso-zeaxanthin can be considered as “pure” antioxidant because it exhibits little or no pro oxidative behaviour, even at high carotenoid concentration and at high oxygen tension (Martin et al., 1999). Acknowledgements

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