Fucoxanthin: A Potential Dietary Neuroprotectant against Brain Aging

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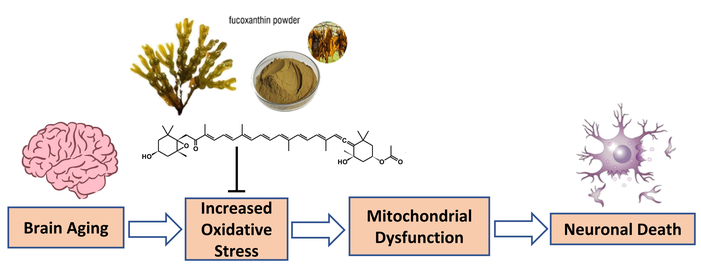
Abstract

Brain aging is associated with excessive production of reactive oxygen species (ROS) causing mitochondrial dysfunction and increasing membrane permeability, leading to neuronal death. DJ1 is an oxidative stress response protein that protects neurons from oxidative damage by scavenging free radicals. Fucoxanthin is a potent marine carotenoid found in brown seaweeds. The effects of fucoxanthin during brain aging are not well studied. We hypothesize that fucoxanthin treatment protects the brain from oxidative stress by increasing the DJ1 protein level. Rat primary hippocampal neurons were cultured with or without fucoxanthin for 6 wk. Cell viability was assessed at 3, 4, 5, and 6 wk, and mitochondrial membrane potential was assessed at 6 wk using propidium iodide (PI) and tetramethylrhodamine methyl ester (TMRM) staining, respectively. Neurons were exposed to hydrogen peroxide to induce oxidative stress. Mitochondrial superoxide and membrane potential were measured using mitoSOX and TMRM, respectively. Middle-aged male Sprague Dawley rats were orally supplemented with fucoxanthin (1 mg/kg, 5 d/w for 4 wk). DJ1 protein was quantified in the hippocampus via immunoblotting. T-test and one-way ANOVA were conducted to compare results among groups. Fucoxanthin prevented age-related mitochondrial dysfunction and neuronal death. Fucoxanthin decreased mitochondrial ROS and preserved mitochondrial membrane potential during oxidative stress. Oral supplementation of fucoxanthin increased DJ1 levels in the hippocampal region of middle-aged rats. In summary, fucoxanthin treatment increases protein levels of DJ1 in the hippocampus and protects mitochondria during ROS challenges in primary hippocampal neurons. Our findings suggest the neuroprotective potential of fucoxanthin against ROS-associated mitochondrial dysfunction during aging.

Background

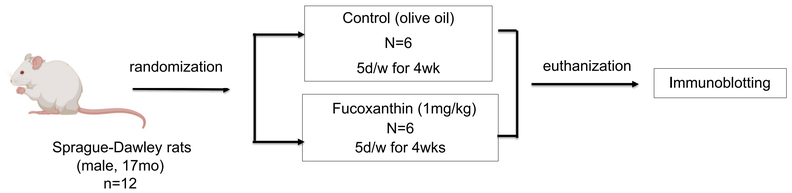


The aging brain is highly susceptible to oxidative damage due to a decline in the antioxidant defense system. Aging also causes mitochondrial respiratory chain dysfunction leading to the accumulation of ROS in the mitochondria[1,2]. Excessive ROS alters mitochondrial permeability causing further damage to the mitochondria, affecting mitochondrial function, and ultimately causing neuronal death[1,2].

DJ1 is an oxidative stress sensor that scavenges free radicals and prevents ROS accumulation[3]. In aged brains, DJ1 protein is inactivated due to modification of its structure and altered energy metabolism is observed in the neuron cultures of DJ1 knockout mice[4,5].

Fucoxanthin is a carotenoid found in brown seaweed with strong antioxidant properties[6]. According to previous studies, fucoxanthin exhibits neuroprotective potential against oxidative stress, inflammation, and neurotoxin by decreasing ROS levels, upregulating endogenous antioxidant enzymes, and preventing apoptosis[7,8].

Methods



Fucoxanthin Supplementation: Middle-aged Sprague-Dawley rats were randomly assigned to control or fucoxanthin groups. The fucoxanthin group received 1 mg/kg fucoxanthin dissolved in olive oil by oral gavage (5 d/w for 4 weeks). The control group received the same amount of olive oil. After 4 weeks animals were euthanized, and brains were collected for further experiments.

Western blot: Immunoblotting technique was used with anti DJ1 antibody to quantify DJ1 protein level in the brain tissue. Target protein abundance was calculated using electrochemiluminescence measurements. Densitometry was performed using ImageJ.

Culture of primary hippocampal neurons: Primary hippocampal neurons were collected from rat feti (Sprague–Dawley, Day 18 of gestation; Harlan, Indianapolis, IN) Carlsbad, CA, USA) and grown in neurobasal medium (0.3 × 106 cells/35 mm plate) supplemented with B-27, glutamine, and antibiotics *in vitro* (DIV).

Time course cell viability test and mitochondrial membrane potential: Primary hippocampal neurons were grown with or without fucoxanthin. At 3, 4, 5, and 6 weeks, viable or dead cells were stained with propidium iodide (PI) (0.5μM in sterile PBS) by adding it to the culture medium for 30 min at 37°C. At 6 weeks, neurons were stained with tetreamethylrhodamine methyl ester (TMRM) (5nM in DMSO) for 30 min at 37°C in the dark. Accumulation of TMRM in the mitochondrial membrane depends on the polarization of the membrane. Micrographs were taken with a Zeiss Axiovert A1 microscope and were analyzed using AxioVision 4.9.

Measurement of mitochondrial membrane potential: Primary hippocampal neurons were treated with fucoxanthin, hydrogen peroxide, or a combination of both and then incubated for 6 hours. Then they were stained with TMRM (5nM in DMSO) for 30 min at 37°C in the dark. Images were taken using a Zeiss Axiovert A1 microscope and TMRM fluorescence densitometry was analyzed using AxioVision 4.9.

Measurement of mitochondrial ROS: Production of mitochondrial ROS was analyzed using MitoSOX Red (Invitrogen). The MitoSOX Red dye is oxidized by superoxide in the mitochondria, emitting red fluorescence. Neurons were stained with 1.25 μM of MitoSOX and incubated for 30 min at 37°C. Fluorescent images were taken with a Zeiss Axio Vert.A1 microscope and analyzed using AxioVision 4.9.

Results

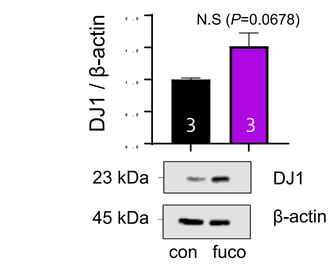


Figure 1. Fucoxanthin increases DJ1 protein levels in the rat hippocampal tissue. In middle-aged Sprague-Dawley rats, oral fucoxanthin supplementation of 1mg/kg for 4 weeks increases protein levels of DJ1 in the hippocampal tissue compared to the control group. 2-tailed t-test

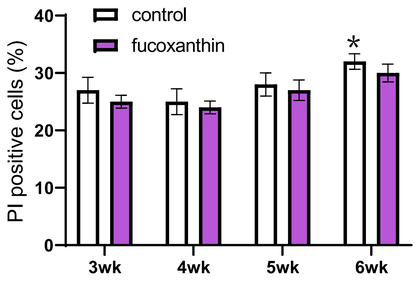


Figure 2. Fucoxanthin prevents aging-associated neuronal death in rat primary hippocampal neurons. PI fluorescent stain permeates dead cells. The number of PI-positive cells increases with age. Treatment with fucoxanthin decreases the number of PI-positive neurons in primary hippocampal culture. *P<0.05, 2-tailed t-test

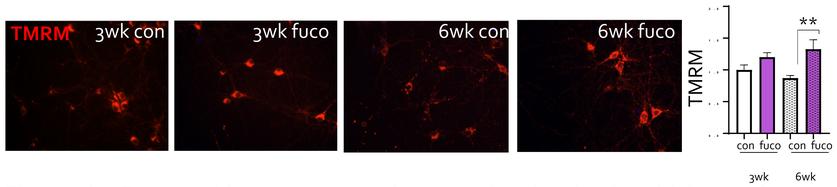


Figure 3. Fucoxanthin prevents aging-associated mitochondrial membrane potential loss in primary hippocampal neurons. Fucoxanthin treatment increases TMRM signal in aged primary hippocampal neurons. At 3 weeks, TMRM signal is similar in control and fucoxanthin groups. Signal decreases at 6 weeks which is retained by fucoxanthin treatment. **P<0.01, 2-tailed t-test

Results

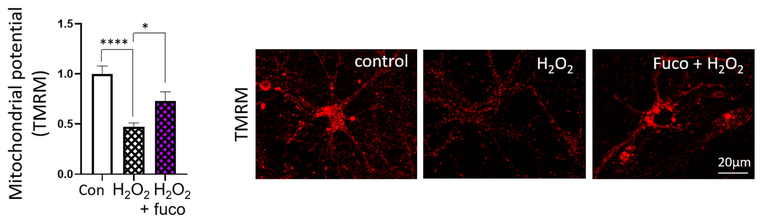


Figure 4. Fucoxanthin prevents mitochondrial membrane potential loss in primary hippocampal neurons induced by oxidative stress. A decrease in TMRM signal after hydrogen peroxide treatment indicates loss of mitochondrial membrane potential. Fucoxanthin prevents the loss of TMRM fluorescence against hydrogen peroxide challenges. * P<0.05 and **** P<0.0001, One-way ANOVA with a Tukey post hoc analysis.

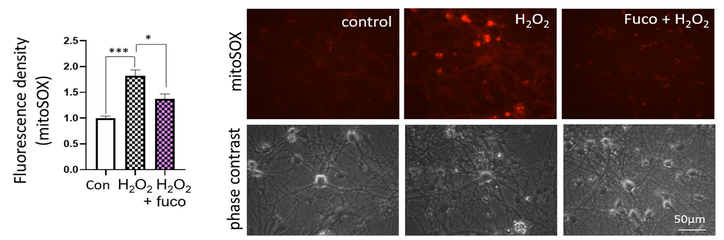


Figure 5. Fucoxanthin prevents the accumulation of mitochondrial superoxide in primary hippocampal neurons induced by oxidative stress. Hydrogen peroxide increases mitoSOX signal, indicating accumulation of mitochondrial superoxide in hippocampal neurons. Treatment with fucoxanthin decreases mitoSOX signal during hydrogen peroxide challenges. * P<0.05 and **** P<0.0001, One-way ANOVA with a Tukey post hoc analysis.

Conclusion

Fucoxanthin may protect neurons from aging-related oxidative stress mitochondrial dysfunction.

References

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