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Supplementation of lycopene attenuates oxidative stress induced neuroinflammation and cognitive impairment via Nrf2/NF-κB transcriptional pathway

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ABSTRACT

Oxidative stress, considered as a major culprit in brain aging, triggers cognitive and memory impairment. Lycopene (LYC), a carotenoid pigment, possesses potent antioxidative, anti-inflammatory, and neuroprotective properties. In the present study, the effects of LYC on oxidative stress-induced cognitive defects and the underlying mechanisms were determined. The behavioral tests, including Y-maze test, locomotor activity and Morris water maze test, revealed that chronic LYC supplementation (50 mg/kg bodyweight per day) alleviated D-galactose induced CD-1 male mice cognitive impairments. LYC ameliorated histopathological damage and restored brain derived neurotrophic factor (BDNF) levels in the hippocampus of mice. LYC also significantly elevated antioxidant enzymes activities and reduced levels of inflammatory cytokines in the D-galactose-treated mice serum. Moreover, LYC treatment activated the mRNA expressions of antioxidant enzymes HO-1 and NQO-1, and downregulated inflammatory cytokines IL-1β and TNF-α in mice hippocampus. Immunohistochemical results also demonstrated that LYC significantly restored the expression of glial cells inflammatory makers Iba-1 and GFAP. Furthermore, LYC attenuated neuronal oxidative damage through activation of Nrf2 signaling and inactivation of NF-κB translocation in a H_2O_2-induced SH-SY5Y cell model. Therefore, these results illustrated that LYC could ameliorate oxidative stress induced neuroinflammation and cognitive impairment possibly via mediating Nrf2/NF-κB transcriptional pathway.

1. Introduction

Oxidative stress and neuroinflammation are associated with diverse chronic neurodegenerative diseases such as Parkinson's disease (PD) or Alzheimer's disease (AD), which usually lead to cognitive decline and neuronal loss (Bao et al., 2014; Zhou et al., 2013). The brain is vulnerable to oxidative damage as it requires a great amount of oxygen, high level of unsaturated lipids, and relative deficiency in anti-oxidative defense mechanisms (Akbaraly et al., 2013). Accumulated evidence suggests that oxidative stress may be a major culprit in brain aging (Harper et al., 2004; Lin and Beal, 2006). Recent studies revealed that chronic inflammations is also an essential causative factor accounting for neurodegenerative diseases (Allison and Ditor, 2014; Chung et al., 2006). The proinflammatory cytokines, such as IL-1β and TNF-α in the brain are considered to lead to neuronal damage and subsequent neuronal loss (Heneka et al., 2015; Marchesi, 2011). It was reported that activated oxidative stress response causes a downstream pathological cascade, which involves activation of NF-κB and expressions of other inflammatory mediators (Karasek, 2004; Lu et al., 2010; Song et al., 1999). Thus, either early prevention of oxidative stress or management of inflammation could ameliorate the chronic neurodegenerative diseases.

Lycopene (LYC), a natural carotene and carotenoid pigment, is abundantly found in red colored fruits and vegetables such as tomato, papaya, pink grapefruit, pink guava and watermelon (Mortensen, 2006), has drawn great attention due to its various bioactivities. LYC is

Abbreviations: AD, Alzheimer's disease; BDNF, Brain derived neurotrophic factor; CAT, Catalase; COX-2, Cyclooxygenase-2; D-gal, D-galactose; GFAP, Glial fibrillary acidic protein; GSH, Glutathione; GSH-PX, Glutathione peroxidase; HE, Hematoxylin-eosin staining; HO-1, Heme oxygenase-1; Iba-1, Ionized calcium binding adaptor molecule-1; IHC, Immunohistochemistry; IL-1β, Interleukin-1 beta; iNOS, Inducible nitric oxide synthase; LYC, Lycopene; MDA, Malonaldehyde; NF-κB, Nuclear factor kappa B; NQO-1, NAD(P)H: quinone oxidoreductase-1; Nrf2, Nuclear factor erythroid-2 related factor 2; PD, Parkinson's disease; Sirt1, Silent mating type information regulation 2 homolog-1; SOD, Superoxide dismutase; TNF-α, Tumor necrosis factor-α

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an unsaturated acyclic carotenoid with 11 linear conjugated and two non-conjugated double bonds. It has been demonstrated that LYC exerts antioxidant and anti-inflammatory effects both *in vitro* and *in vivo* and possesses blood brain barrier permeability (Jain et al., 1999; Lee et al., 2012; Marcotorchino et al., 2012). It has also been reported that the higher levels of serum carotenoid pigments such as lycopene, lutein and zeaxanthin lowered the risk of neurodegenerative diseases (Min and Min, 2013).

The current study was aimed to uncover the protective effects of dietary supplementation of LYC on D-Galactose (D-gal) induced oxidative stress and neuroinflammation in CD-1 mice. D-galactose (D-gal) is a reductive sugar which is abundantly present in milk products, fruits and vegetables (Gropper et al., 2000). Several studies have suggested that chronic systemic administration of D-gal could be used as an oxidative stress model, which can cause cognitive disorders and aging (Cui et al., 2006; Hsieh et al., 2009; Lei et al., 2008). The effects of LYC on D-gal-induced memory impairment and neuronal damage were determined via behavioral tests. Besides, to evaluate the effects of LYC on oxidative stress and neuroinflammation, the levels of antioxidant enzymes and inflammatory cytokines were determined. Furthermore, the antioxidant and anti-inflammatory mechanisms of LYC in were examined by a H_2O_2 pretreated SH-SY5Y neuronal cell model.

2. Materials and methods

2.1. Reagents and antibodies

Lycopene and D-galactose were purchased from Sigma Aldrich, St Louis, MO, USA. The malonaldehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), glutathione (GSH), and catalase (CAT) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TNF-α and IL-1β levels were detected using ELISA kits (Mouse TNF-α kit, Mouse IL-1β kit, Xinle Biology Technology, Shanghai, China). Iba-1 (ab178847), GFAP (ab10062) were purchased from Abcam, Inc., MA, USA. HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, USA.

2.2. Animals

CD-1 male mice (6 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animals were afterwards housed in rectangular cages in a controlled atmosphere with a 12 h light/dark cycle. Commercial basal chow diet (AIN-93M, purchased from TROPHIC Animal Feed High-tech Co., Ltd., Nantong, China) and distilled water were provided ad libitum.

After one week of adaptation, a total of 40 mice were randomly divided into 4 groups (n = 10 mice/group). (a) Control group (Con): Mice fed with commercial basal chow diet (AIN-93M) and treated with 0.9% saline (intraperitoneally injection, i.p.); (b) Lycopene group (LYC): Mice treated with commercial basal chow diet (AIN-93M) mixed with LYC (0.033% w/w), which is approximate 50 mg/kg bodyweight per day LYC supplementation for each mice; (c) D-galactose group (D-gal): Mice administered with D-galactose (150 mg/kg/day, i.p); (d) D-galactose plus lycopene group (D-gal + LYC): Mice treated with D-galactose (150 mg/kg/day, i.p.) and simultaneously fed with commercial basal chow diet (AIN-93M) mixed with LYC (50 mg/kg). Con group and LYC group were treated with 0.9% saline. Lycopene was mixed in commercial basal chow diet (AIN-93M, purchased from TROPHIC Animal Feed High-tech Co., Ltd. Nantong, China). The experiment was carried out for a period of 8 weeks. All of the experimental procedures followed the Guide for the Care and Use of Laboratory Animals: Eighth Edition, ISBN-10:0–309-15396-4, and the animal protocol was approved by the animal ethics committee of Northwest A & F University. All the surgery was performed under anesthesia and efforts were made to minimize suffering.

2.3. Behavioral tests

After 8 weeks' treatment of D-gal and LYC, the behavioral study of the mice (n = 10/group) was performed using Y-maze test and locomotor activity test. After a day's rest, Morris water maze tests were conducted.

2.3.1. Y-maze test

To assess basic mnemonic processing (by percentage of alternation) and exploratory activity (by total number of arm choices), each mouse was placed at the center of a black Y- maze (20 cm long, 4 cm wide, 40 cm high) and allowed free exploration of the three arms for 8 min. The number of total arm entries and the sequence of the arm entries were recorded and three consecutive choices were defined as an alternation.

2.3.2. Locomotor activity test

The locomotor activity of each mouse was implemented in a locomotor monitoring box (25 cm long, 25 cm wide, 25 cm high) for 6 min. Total distance of mice spontaneous movement and average speed were recorded by a computerized video-tracking system (SuperMaze software, Shanghai Xinruan Information Technology Co., Ltd, China).

2.3.3. Morris water maze test

Spatial learning and memory was analyzed using Morris water maze test. The apparatus is made up of a circular water tank with a diameter of 100 cm and a height of 40 cm. A transparent platform (10 cm in diameter, 20 cm in height) was placed hidden 1 cm below the water surface at one quadrant. The mice were allowed to receive four training periods per day for 4 consecutive days. For each trial, the latency to escape from the water of each rat was calculated. On day 5, probe test was performed and the mice were allowed to swim freely for 60 s with the platform removed. The latency to the platform, the time that was spent in the target quadrant and three non-quadrants (right, left, and opposite) during swimming, and the number of platform crossings was measured. All the data was recorded via visual tracking system (SuperMaze software, Shanghai Xinruan Information Technology Co., Ltd, China).

2.4. Preparation for brain and serum samples

Mice were sacrificed after anesthesia with 10% chloral hydrate (dissolved in physiological saline, 4 ml/kg body weight). Serum samples were separated from orbital eye bleeding under anesthesia which were stored at −80 °C for subsequent experiments. In each group, five mice were used for the biochemical analysis and another five mice were used for histopathological examination and immunohistochemistry tests. The brains of the mice were carefully and quickly removed and washed with cold physiological saline. The hippocampus were immediately separated from the cerebrums on a cold plate and stored in liquid nitrogen for the biochemical analysis and some mRNA expression. The brains of the mice were stored in 4% phosphate-buffered paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4) for histopathological examination and immunohistochemistry tests.

2.5. Biochemical analysis

The hippocampi of the mice were homogenized in tissue total protein lysis buffer (1 g tissue in 9 mL lysis buffer). The homogenate was then centrifuged at 4000 g for 10 min and the supernatant was used for the subsequent tests. The concentration of protein was measured using a commercial BCA protein assay kit and using bovine serum albumin (BSA) as a standard. The levels of GSH, MDA and CAT, and the activities of SOD and GSH-PX in the hippocampus homogenate and serum were assayed respectively using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). TNF-α and IL-1β in serum were analyzed