Neuroprotective effects of lycopene pretreatment on transient global cerebral ischemia-reperfusion in rats: The role of the Nrf2/HO-1 signaling pathway

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Abstract. The present study aimed to investigate the neuroprotective effect of lycopene in a mouse model of bilateral common carotid artery occlusion (BCCAO) and the role of the Nrf2/HO-1 signaling pathway. A total of 60 male C57BL/6 mice, aged 12 weeks and weighing 20-24 g, were used in the present study. The mice were randomly assigned to three groups: Control, BCCAO and BCCAO + lycopene. The neurological score was assessed 24, 48 or 72 h following BCCAO. Hematoxylin and eosin staining, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) were performed to detect neuronal death and survival. The production of glutathione (GSH) and reactive oxygen species were detected to investigate the oxidative stress. The expression levels of nuclear factor erythroid 2-related factor (Nrf2) and Heme oxygenase-1 (HO-1) were determined by western blotting. Lycopene significantly improved the neurological score in the BCCAO mice. It attenuated neuronal apoptosis, as indicated by TUNEL staining, and attenuated the oxidative stress induced by global ischemia. Lycopene increased the expression levels of Nrf2 and HO-1, indicating that the Nrf2/HO-1 signaling pathway may be involved in the neuroprotective effect of lycopene. The present study revealed that lycopene protects the brain from global ischemic injury, which is associated with its antiapoptotic effect and the activation of the Nrf2/HO-1 signaling pathway.

Introduction

Ischemic stroke is currently a leading cause of cerebrovascular disease worldwide, and exhibits a high morbidity and mortality among patients (1). Ischemic stroke is induced by a transient or permanent occlusion in the cerebral vessel, resulting in neuronal death and associated behavioral deficits, including sensorimotor dysfunction, spatial orientation disorder, and learning and memory impairment (2-4). In addition, the mechanisms underlying stroke include oxidative stress, blood-brain barrier dysfunction, neuronal apoptosis and inflammation (5,6). Although tissue-type plasminogen activator is used clinically and remains the only FDA-approved treatment for ischemic stroke, it is not so effective for all patients and only a small number of patients recover as a result of the reperfusion injury and a narrow 3 h time-window for safe administration (7). Therefore, other effective therapeutic agents are required to assist the patients with their diseases.

Lycopene, a member of the carotenoid family, is found predominantly in tomatoes and other red colored fruits (8). It has been previously reported that lycopene has several biological functions in various diseases. Lycopene protects the cell from lipid peroxidation and oxidative DNA damage as a highly efficient antioxidant (8,9). In addition, lycopene exhibits other properties, including antiapoptosis (10), anti-inflammatory (11,12), antiamyloid (13), anti-ischemia (14), and antitumor properties (15). Since lycopene has a high liposolubility, it can cross the blood-brain barrier (16). It has also been demonstrated that lycopene is beneficial for certain neurological disorders, including Alzheimer's disease (17,18). Therefore, lycopene is potentially beneficial in other brain diseases and the present study set out to investigate this.

Oxidative stress is important in ischemic stroke, characterized by a dramatic increase in reactive oxygen species (19). In normal cells, the antioxidant system protects cells from various oxidative stresses. Antioxidant/electrophile response element (ARE)-regulated phase II detoxifying enzymes and antioxidants are one of the predominant antioxidant pathways involved in attenuating increased oxidative stress and maintaining the redox status in several tissues and organs (20). Heme oxygenase-1 (HO-1) is an ARE-regulated enzyme and antioxidant, which is regulated by the redox-sensitive transcription factor, nuclear factor erythroid 2-related factor (Nrf2) (21). The function of HO-1 is to catalyze heme to biliverdin, carbon monoxide and iron. It has been previously reported that Nrf-2...
activation protects the neurons from ischemia (22). Under physiological conditions, Nrf2 is located in the cytosol and binds to Kelch-like ECH-associated protein 1 (Keap1). In response to oxidative stress, Nrf2 dislocates from Keap1 and translocates to the nucleus (23,24), where it forms a heterodimer with its obligatory partner, Maf, and binds to the ARE sequence to activate the transcription of numerous antioxidant and electrophile detoxification genes, including HO-1, NAD(P)H:quinone oxidoreductase 1 and glutamate-cysteine ligase (25).

The present study aimed to investigate whether lycopene exerts a neuroprotective effect on the ischemic brain in a bilateral common carotid artery occlusion (BCCAO) model. If so, the present study aimed to determine whether it regulates Nrf2/HO-1 signaling in this ischemic model.

Materials and methods

Animals. A total of 60 C57BL/6 mice, aged 12 weeks and weighing 20-24 g, were used in the experiments and were provided by the Experimental Animal Center of the Tianjin Medical University (Tianjin, China). The mice were maintained in cages under a controlled-light environment (12 h light/dark cycles) and were allowed free access to a rodent diet and tap water. The present study was approved by the Ethics Committee of Tianjin Medical University. All animals used in this study were cared for in accordance with the Guidance for the Care and Use of Laboratory Animals published by the United States National Institute of Health.

Establishment of global cerebral ischemia. BCCAO was used as a model of global cerebral ischemia, as previously reported (26). Surgical operation was performed by an individual in a blinded manner. The mice were anesthetized with 3% isoflurane (Baxter, Deerfield, IL, USA). Following induction, the concentration of isoflurane was maintained at 1.5%. Isoflurane was administered via a face mask, which was constructed to fit over the animals’ frontal area. A midline incision was made to the region between the neck and sternum to expose the trachea. The right and left common carotid arteries were located lateral to the sternocleidomastoid and were carefully separated. Cerebral ischemia was induced by clamping each of the arteries with two miniature artery clips. Following 20 min of cerebral ischemia, the clips were removed from each artery to allow for the reperfusion of blood through the carotid arteries. Sham-operated mice underwent the identical surgical procedure without artery occlusion. During the surgical procedure, the pericranial temperature was monitored using a temperature probe and maintained at 37.0-37.5°C using a heating pad. Following surgery, the animals were placed in a warm environment (30-33°C) to avoid biased results due to hypothermia.

Drug administration. For the BCCAO + lycopene group, lycopene was intraperitoneally administered at a dose of 20 mg/kg for seven consecutive days prior to surgery. The mice in the sham group and BCCAO group were injected solely with an equal concentration of dimethyl sulfoxide (DMSO). Lycopene was dissolved in 2% DMSO.

Neurological tests. The treated mice were allowed to recover for 24 h prior to subsequent tests. The mice were subjected to a modified neurological examination designed to detect motor deficits. Briefly, the mice were placed on a 10-20 cm screen (grid size 0.2x0.2 cm), which can be rotated from 0° (horizontal) to 90° (vertical). The mice were placed on this screen, which was in a horizontal position, and the screen was then rotated into the vertical plane. The duration for which each mouse was able to hold on to the vertical screen was recorded up to a maximum of 15 sec (corresponding to a maximum of three points). Next, the mouse was placed at the center of a horizontal wooden rod (diameter, 1.5 cm), and the duration that the mouse was able to remain balanced on the rod was recorded up to a maximum of 30 sec (corresponding to a maximum of three points). Finally, a prehensile traction test was performed. The duration that the mouse was able to cling to a horizontal rope was recorded up to a maximum of 5 sec (corresponding to a maximum of three points). From these tests, a total motor score (TMS; nine possible points) was calculated. The neurological assessments were performed at 24, 48 or 72 h post-reperfusion by an observer in a blinded manner. The TMS has been shown previously to be an accurate method for evaluating global cerebral ischemic injury in mice (27).

Hematoxylin and eosin (HE) staining. Neuronal damage was assessed using HE staining (Beyotime Institute of Biotechnology, Shanghai, China). On day 3 following the induction of ischemia, the animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally; Sigma-Aldrich, St. Louis, MO, USA) and transcardially perfused with 4% phosphate-buffered paraformaldehyde, following a flush with 0.1 M phosphate-buffered saline (PBS). The brains were removed, post-fixed at 4°C in 4% paraformaldehyde overnight and then sectioned on a freezing microtome. The brains were sectioned backward from the optic chiasm into six consecutive sections (12 µm), which included the dorsal hippocampus, and were stained with HE. The pyramidal neurons of the CA1 region were examined.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). The tissue sections were placed on slides and incubated with TUNEL reaction mixture (Roche Diagnostics GmbH, Mannheim, Germany), including enzyme solution (terminal deoxynucleotidyl transferase) and tetramethylrhodamine-labeled TUNEL-positive nucleotides, in a dark humidified chamber for 1 h at 37°C, followed by a final wash for 3x10 min with PBS and then covered with water-based mounting medium (National Diagnostics, Atlanta, GA, USA). The captured images were viewed and analyzed using laser scanning confocal microscopy (FV1000; Olympus, Tokyo, Japan).

Western blot analysis. Proteins were extracted following brain tissue homogenization in radioimmunoprecipitation acid buffer (EMD Millipore, Billerica, MA, USA). The total protein content was determined using a bicinchoninic acid protein assay. The protein samples (50 µg) were separated by electrophoresis on SDS-PAGE gels (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and were transferred onto polyvinylidene difluoride membranes (EMD Millipore). The membranes were blocked with 5% non-fat milk at room
temperature for 2 h and were incubated overnight with the appropriate primary antibodies. The antibodies used were rabbit polyclonal anti-Nrf2 (1:200; cat. no. sc-13032; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit polyclonal anti-HO-1 (1:200; cat. no. sc-10789; Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-Histone1 (1:1,000; cat. no. ab4270; Abcam, Cambridge, MA, USA), and mouse monoclonal anti-β-actin (1:5,000; cat. no. sc-47778; Santa Cruz Biotechnology, Inc.). Following extensive rinsing with Tris-buffered saline, containing 0.1% Triton X-100 buffer, the membranes were incubated with mouse anti-rabbit (cat. no. sc-2357) and goat anti-mouse (cat. no. sc-2005) horseradish peroxidase-conjugated secondary antibodies (1:2,000; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The membranes were developed and a bar graph was produced to depict the ratios of semi-quantitative results obtained by scanning reactive bands and quantifying the optical density using Image Lab version 4.0 software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis. All statistical analyses were performed using SPSS 11.0 for Windows software (SPSS, Inc., Chicago, IL, USA). All values, with the exception of TMS, are presented as the mean ± standard error of the mean, and were analyzed using a one-way analysis of variance. Between-groups, differences were detected based on post-hoc Student-Newman-Keuls tests. The TMS are expressed as the medians and were analyzed using the Kruskal-Wallis test. P<0.05 was considered to indicate a statistically significant difference.

Results

Neurological score. As shown in Fig. 1, the neurological score in the BCCAO group markedly decreased compared with that in the sham group (P<0.05) at 24, 48 and 72 h following the induction of ischemia. Lycopene treatment ameliorated the injury and the neurological score was increased compared with that in the BCCAO group (P<0.05).

HE staining. A total of 3 days following reperfusion, the number of viable neurons in the CA1 region was markedly decreased in the BCCAO group. Lycopene treatment significantly reduced the neuronal degeneration in the CA1 region compared with that in the BCCAO group (Fig. 2).

TUNEL. As shown in Fig. 3, ischemia induced a marked neuronal apoptotic response compared with the sham group. The survival of the neurons was markedly increased when lycopene was administered, suggesting that lycopene treatment attenuated the apoptosis of neurons, as indicated by the decrease in the number of TUNEL-positive neurons in the CA1 region.

Oxidative stress. Global cerebral ischemia induced a dramatic decrease in the production of GSH and a significant increase in the production of reactive oxygen species (ROS). When lycopene was administrated, the production of GSH was increased (P<0.05) and the production of ROS was decreased (P<0.05), indicating that lycopene protects the ischemic brain from oxidative stress (Fig. 4).
Effect of lycopene on the expression of Nrf2 and HO-1. As shown in Figs. 5 and 6, the expression levels of nuclear and total Nrf2 and HO-1 in the hippocampus were detected by western blotting. The nuclear and total Nrf2 were markedly upregulated in the lycopene treatment group. In addition, lycopene significantly upregulated the expression of HO-1.

Discussion

The results of the present study demonstrated that lycopene preconditioning has a neuroprotective effect in cerebral ischemia-reperfusion in mice. Lycopene preconditioning significantly improved the TMS and reduced neuronal death.
following cerebral ischemia-reperfusion. It was revealed that lycopene pretreatment induced an antiapoptotic effect and an antioxidative stress effect, which is demonstrated by its ability to increase the production of GSH and to decrease the production of ROS. In addition, lycopene activated the expression of Nrf2 and HO-1 in this global ischemic model.

Pyramidal neurons in the hippocampal CA1 region are particularly vulnerable to ischemia. This region undergoes delayed neuronal death, often reported as apoptosis in collaboration with DNA fragmentation (28). In the present study, a model of BCCAO was established and it was subsequently determined that lycopene pretreatment protected the brain from the ischemic injury, which is associated with its antiapoptotic effect and its antioxidative stress effect.

Neuronal apoptosis is an important pathological process of ischemic stroke (29,30). Rabuffetti et al (31) demonstrated that the inhibition of apoptosis reduces ischemic injury. Although two pathways of apoptosis, extrinsic and intrinsic, have been recognized (32), the final phase of apoptosis execution, which
includes activation of executioner caspases (e.g. caspase 3), is shared by each of these pathways (33). Lycopene, a member of the carotenoid family, is found predominantly in tomatoes and other red colored fruits (8). It has been reported that lycopene protects against apoptosis in hypoxia/reoxygenation-induced H9C2 myocardioblast cells (34). In addition, He et al (35) reported that lycopene attenuates inflammation and apoptosis in postmyocardial infarction remodeling. The protective effect of lycopene in retinal ischemia/reperfusion injury has been demonstrated. It has been suggested that lycopene reduces the apoptosis of cells in the ganglion cell layer (36). The present study is in agreement with these studies and the results suggested that lycopene attenuated neuronal apoptosis in global ischemic brain.

Oxidative stress is also significant in ischemic brain injury (37). In ischemia/reperfusion injury, ROS is markedly produced and the endogenous antioxidant system cannot eliminate many of them. As a result, ROS leads to lipid peroxidation and DNA damage. It has been suggested that lycopene protects pancreatic acinar cells against severe acute pancreatitis (38). Additionally, lycopene prevents experimental priapism against oxidative damage, as reported previously (39), and attenuates oxidative stress in fructose-drinking insulin resistant rats (40). Consistent with these studies, the present study suggested that lycopene attenuates oxidative stress induced by global ischemia in the brain.

HO-1 is a rate-limiting enzyme, catalyzing the degradation of heme into carbon monoxide, biliverdin and ferritin (41). HO-1 is regulated by the transcription factor Nrf2 at the transcriptional level (42). Under physiological conditions, Nrf2 is located in the cytosol by binding to Keap1 (43). In the presence of ROS, Nrf2 is released from Keap1 and translocates into the nucleus, activating the transcription of HO-1. In the present study, brain ischemia/reperfusion injury leads to a dramatic increase in the generation of ROS. Consequently, nuclear Nrf2 was increased and HO-1 was upregulated following ischemia-reperfusion injury. In addition, lycopene pretreatment significantly induced an increase in the expression levels of Nrf2 and HO-1.

In conclusion, these findings suggested that lycopene provided significant neuroprotection in mice subjected to global cerebral ischemia by inhibiting neuronal apoptosis and attenuating oxidative stress, which is associated with the activation of Nrf2/HO-1 signaling.

References


