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Research report

Preventive treatment of astaxanthin provides neuroprotection through suppression of reactive oxygen species and activation of antioxidant defense pathway after stroke in rats

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ABSTRACT

Astaxanthin, a natural antioxidant carotenoid, has been shown to reduce cerebral ischemic injury in rodents. However, there have not been any studies specifically addressing whether preventive administration of astaxanthin can protect against cerebral ischemia. The purpose of this study was to examine whether pretreatment of astaxanthin can protect against ischemic injuries in the adult rats. The rats were pre-administered intragastrically with astaxanthin for seven days (once a day), and middle cerebral artery occlusion was performed at 1 h after the final administration. It was found that astaxanthin prevented neurological deficits and reduced cerebral infarction volume. To evaluate the mechanisms underlying this protection, brain tissues were assayed for free radical damage, antioxidant gene expression, cell apoptosis and regeneration. The results showed that the mechanisms involved suppression of reactive oxygen species, activation of antioxidant defense pathway, and inhibition of apoptosis as well as promotion of neural regeneration. Astaxanthin did not alter body weights and the protective effect was found to be dose-dependent. Collectively, our data suggest that pretreatment of astaxanthin can protect against ischemia-related damages in brain tissue through multiple mechanisms, hinting that astaxanthin may have significant protective effects for patients vulnerable or prone to ischemic events.

1. Introduction

Stroke remains one of the most devastating of all neurological conditions, and worldwide it is currently the second leading cause of death in those over 60 years old and the fifth leading cause of death in people aged 15–59 years old (Feigin et al., 2009). In China, the annual stroke mortality rate is approximately 157 per 100 000, which has exceeded heart disease to become the leading cause of death and adult disability. Stroke has been reported to cause approximately 116 deaths in population of 100 000 in cities and 111 deaths in rural areas, and there are 2.5 million new stroke cases (Chen, 2008). This poses a major public health and economic burden in China. The cost for stroke care by the government-funded hospitals was 1.17 billion RMB in 2003 and 8.19 billion in 2009 (117% increase annually). Now the annual cost of stroke care in China is approximately 40 billion RMB, 10 times higher than the care of cardiovascular diseases (Hu and Gong, 2003; M.o.H. PRC, 2010).

Ischemic stroke, as the most common type of stroke in China, accounts for 43% to 79% of all strokes, which has a higher percentage (18%–47%) than that in Western countries. The China Acute Cerebrovascular Events Registers (CACER-I) reported that ischemic stroke cases were constantly increasing and creating a serious public health problem in China (Liu et al., 2007). Cerebral ischemia always triggers an almost immediate loss of oxygen and glucose to the cerebral tissue and ultimately causes irreversible neuronal injuries in the ischemic core within minutes of the onset (Dirnagl et al., 1999). Acute responses of brain tissue to cerebral ischemia and its chronic pathogenic progression involve many different pathways, but increasing evidence has shown that oxidative stress and inflammation are established and important mechanisms underlying the pathogenesis of stroke (Muir et al., 2007).

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The redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) has been found to protect neurons against oxidative damage triggered by ischemic stroke (Ishii et al., 2004; Ishii et al., 2000; Kaspar et al., 2009; Kensler et al., 2007). Under normal conditions, Nrf2 is kept in the cytoplasm by Kelch like-ECH-associated protein 1 (KEAP1), which suppresses the activity of Nrf2 (Itoh et al., 1999). While, under oxidative stress, the Nrf2–Keap1 complex is dissociated and Nrf2 travels to the nucleus where it binds to antioxidant response element (ARE) and initiates transcription of downstream antioxidant genes, such as HO-1 (Heme oxygenase-1) (Jarmi and Agarwal, 2009; Wang and Doré, 2007) and NQO1 (NAD(P)H quinone oxidoreductase 1) (Venugopal and Jaiswal, 1996). This implicates that Nrf2–ARE antioxidant pathway may serve as a therapeutic target for neurovascular protection in stroke.

Astaxanthin (ASX), a naturally occurring biological antioxidant, has been reported to improve cellular defenses against oxidative stress (Amar et al., 2001; Bell et al., 2000; Nakano et al., 1999; Palozza and Krinsky, 1992). It is found in a variety of living organisms, including microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans, and the feathers of some birds. While ASX is a primary component of coloration, this pigment has other physiological roles, such as protective effects against ultraviolet (UV) light-induced damage (Gorton and Vogelmann, 2003; Gorton et al., 2001) and oxidative stress (Kobayashi, 2000), as well as increasing resistance to physical and chemical stressors (Chien et al., 2003; Merchie et al., 1998; Pan et al., 2003). Although ASX has a molecular structure similar to that of β-carotene, it has 13 conjugated double bonds, in contrast to 11 in β-carotene. This gives it significantly greater antioxidant capacity (Shibata et al., 2001), ten times higher than that of β-carotene (Naguib, 2000). Moreover, ASX has been found to up-regulate the activity of Nrf2–ARE pathway and protect the cells from oxidative stress (Tripathi and Jena, 2010; Li et al., 2013). However, there have not been any studies specifically addressing whether preventive administration of ASX can protect against ischemic brain injuries.

To answer this question, the current experimental protocol was designed to prove whether preventive administration of ASX can protect the rats from damages induced by MCAO (middle cerebral artery occlusion) surgery, and the possible mechanisms underlying this protection were further explored.

2. Materials and methods

2.1. Animals

The selected animals were male SD (Sprague-Dawley) rats and aged of 3 months old with a mass from 250 g to 300 g. They were of SPF (specific pathogen free) grade and obtained from Institute of Laboratory Animals of Sichuan Academy of Medical Sciences & Sichuan Provicinal People's Hospital (Sichuan, China). The animal certification number was SCXK2008-24. The animals were housed in cages at 24 ± 2 °C with a 50%–60% relative humidity in a 12 h light/dark cycle for at least 1 week before the experimental grouping and fed a normal diet (Lab MR, NOSAN, Ybkohama, Japan). Water was supplied *ad libitum*.

All animal experimental protocol and care were performed in accordance with the standards established by the Institutional Animal Care and Use Committee of Chongqing Medical University.

2.2. Experimental grouping

In this experiment (as shown in Fig. 1), the SD rats were randomized into 5 groups: sham operation group (SOG), model group (MG), positive control group (PCG), high ASX group (HAG) and low ASX group (LAG), with each group having eight animals. The SOG rats were administrated with groundnut oil by means of intragastric (i.g.) administration (10 mg/kg) for 7 days. At 1 h after the final administration, the CCA (common carotid artery) and ICA (internal carotid artery) of rats were separated but not occluded. The MG rats were also administrated with groundnut oil by i.g. administration (10 mg/kg) for 7 days, and the model was made by occlusion of the middle cerebral artery (MCAO) at 1 h after the final administration. The PCG rats were administered with edaravone (10 mg/kg) by intraperitoneal injection (i.p.) for 7 days, while the HAG and LAG rats were respectively administered with high levels of astaxanthin (dissolved in groundnut oil, 10 mg/kg) and low levels of astaxanthin (dissolved in groundnut oil, 5 mg/kg) by i.g. for 7 days. At 1 h after the final administration, all the three treated groups were made by occlusion of the middle cerebral artery (MCAO). Each MCAO model rat was undergone 2 h of ischemia and 24 h of reperfusion.

2.3. MCAO surgery

The MCAO surgery was performed as described by Longa et al. (Longa et al., 1989). In brief, the animals were injected with apomorphine (1 ml/kg) by intramuscular injection (i.m.) to inhibit saliva secretion and anesthetized with chloral hydrate (300 mg/kg, i.p.). The anesthetized animals were placed in supine position on the surgical table, and the body core temperature was maintained at 37 °C during the following surgical operations. The rat's left common carotid artery (CCA) was firstly exposed via a midline pretracheal incision, and carefully separated from the adjacent veins and sympathetic nerves. Then, the ECA (external carotid artery) and its branches were isolated and ligated. A 3–0 nylon suture with a blunted tip was introduced into the ICA (internal carotid artery) through the ECA stump and advanced to the anterior cerebral artery to occlude the MCA (middle cerebral artery). After occluding the MCA for 2 h, the operator carefully removed the suture to restore blood flow and then sutured the skin to allow the animal to wake up.

2.4. Neurological function evaluation

Once the animals woke up, the neurological status of each rat was immediately evaluated by an observer who was blind to the experimental grouping. The Bederson scores of 0–3 were used to evaluate neurological function (Bederson et al., 1986). Briefly, the rat was suspended with its tail at about one meter above the floor. If the rat extended both forelimbs toward the floor that suggested it had no neurological deficits and was assigned a score of 0 point. If the observed rat exhibited flexion of the left limb toward the body and/or rotation of the left shoulder and limb medially, it was regarded as neurologically impaired. When the animal was observed to display the above-mentioned posture, it would be placed on a sheet of soft plastic-backed paper that could be gripped by its claws. Lateral pressure would be applied from behind the shoulders to slid the forelimbs gently to the left and then to the right. Resistance to sliding in both directions was scored 1, while a decreased resistance to the lateral push from the right side was scored 2, and spontaneous anti-clockwise circling or left-sided tumbling or unmoving was scored 3. For each rat, the neurological examination should be performed in 3–5 min and the neurological evaluation was again performed at 24 h after reperfusion.

2.5. Infarction area measurement

After experiencing 24 h of reperfusion and completion of neurological evaluation, all the animals were anesthetized with chloral hydrate (300 mg/kg, i.p.) and sacrificed by drawing blood from abdominal aorta. Serums were gathered through deposi-