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Protective role of luteolin against cognitive dysfunction induced by chronic cerebral hypoperfusion in rats

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ABSTRACT

Chronic cerebral hypoperfusion, a mild ischemic condition, is associated with the cognitive deficits of Alzheimer's disease (AD). Luteolin, a polyphenolic compound found in foods of plant origin, belonging to the flavone subclass of flavonoids, has been shown to possess antioxidant, anti-inflammatory and antitumorigenic properties. In the present study, the effects of luteolin on chronic cerebral hypoperfusion-associated neurocognitive pathologies were investigated by using rats with permanent bilateral common carotid artery occlusion, a rat model of chronic cerebral hypoperfusion. As expected, we found that luteolin could attenuate cognitive dysfunction in chronic cerebral hypoperfused rats, as assessed using Morris water maze tests. Daily oral administration of luteolin (50, 100 and 200 mg/kg) significantly scavenged oxygen free radicals, enhanced antioxidant potential, decreased the lipid peroxide production and suppressed inflammatory reaction in the cerebral cortex and hippocampus induced by chronic cerebral hypoperfusion. Meanwhile, the results indicated that cerebral hypoperfusion activated nuclear factor-κB (NF-κB), increased the expression of β-site amyloid precursor protein cleaving enzyme (BACE1), as well as elevated amyloid beta (Aβ) levels in the cortex and hippocampus. However, long-term administration of luteolin significantly down-regulated the expression of NF-κB and BACE1, accompanied by diminishing the deposition of Aβ. Our results suggest a potential therapeutic use of luteolin for cerebral hypoperfusion associated cognitive dysfunction in AD.

1. Introduction

Alzheimer's disease, one primary type of dementia, is classified as a neurodegenerative disease. Recent research indicates that neurovascular dysfunction contributes to cognitive decline and neurodegeneration in AD (Zlokovic, 2005), especially in the early stages, when chronic cerebral hypoperfusion increases AD-associated cognitive decline. Evidence indicated that microvascular degeneration and chronic cerebrovascular hypoperfusion are characteristic of AD (de la Torre, 1999, 2000; Royall, 2002). As a major contributor to cognitive decline, chronic cerebral hypoperfusion in dementias often precedes the neurodegenerative changes, which are not merely a consequence but rather a pathogenic factor. The classical model always used to investigate the cognitive and histopathologic consequences of chronic cerebral hypoperfusion is permanent bilateral occlusion of the common carotid arteries (2-VO) of rats (Farkas and Luiten, 2001; Farkas et al., 2007).

Previous studies have demonstrated that chronic cerebral hypoperfusion could induce the generation of reactive oxygen species (ROS) and activate the inflammatory glial cells (de la Torre and Aliev, 2005; Wang et al., 2007), thereby leading to various inflammatory reactions. Furthermore, growing data demonstrated that the formation of ROS and the immune inflammatory reaction contribute to the progression of AD (Coyle and Puttfarcken, 1993; Bradt et al., 1998; Colton et al., 2000; Combs et al., 2000).

Research evidenced that oxidative stress and inflammation could result in amyloid-β (Aβ) generation (Agostinho et al., 2010; Candore et al., 2010). The accumulation of Aβ peptide into amyloid plaques in the extracellular brain parenchyma is the central pathological event in AD. Aβ is generated by the β-amyloid precursor protein (APP) by β- and γ-secretases. The β-secretase (β-site amyloid precursor protein cleaving enzyme, BACE1) cleaves the ectodomain of APP, producing an APP C-terminal fragment. This fragment is further cleaved within the transmembrane domain by the β-secretase to generate Aβ peptides with different C-terminal variants, predominantly $Aβ_{40}$ and $Aβ_{42}$

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(Selkoe, 2001; Guglielmotto et al., 2012). Evidence has shown that mice deficient in BACE1 have normal phenotype and abolished Aβ generation suggesting that it might be an attractive target for therapy (Luo et al., 2001).

NF-κB exists in a latent form bound to an inhibitory protein κB (IκB) in the cytosol. The various stimuli, such as oxidative stress, mitogens, apoptotic mediators, and bacterial products (Ghosh et al., 1998; Siebenlist et al., 1994), can lead to degradation of IκB that will unmask the nuclear localization signal of NF-κB. The activated transcription factor is then translocated to the nucleus and can interact with DNA. The important roles of NF-κB have been proposed during neuronal development and neurodegeneration (O'Neill and Kaltschmidt, 1997). It was reported that BACE1 promoter contains NF-κB binding elements (Bourne et al., 2007) and the increased BACE1 expression exacerbated Aβ levels via NF-κB-dependent pathways (Buggia-Prevot et al., 2008).

Flavonoids are a large group of plant secondary metabolites that share a basic phenylbenzopyrone feature and are found in all vascular plants where they occur in several structurally and biosynthetically related classes (Coleta et al., 2008). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health and, they have been reported to have antiviral, anti-inflammatory, antitumor and antioxidant activities. Luteolin, a common flavonoid found in high concentrations in celery and green pepper, has been shown to play a role in the human body possibly as antioxidant and anti-inflammatory (Jang et al., 2008; Zhao et al., 2012). Our previous study has shown that luteolin has a potential effect to attenuate diabetes-associated cognitive decline in rats via modulating the oxidative and choline esterase activities (Liu et al., 2005). In the current study, we aimed to further investigate the protective effect of luteolin on the cognitive dysfunction induced by chronic cerebral hypoperfusion and the possible underlying mechanisms of these effects.

2. Materials and methods

2.1. Animals

One hundred male Sprague–Dawley rats (250–300 g) of 10–12 weeks of age were used in the study. The rats were randomly assigned to 5 groups, that were 20 rats per group. The specification of the cages is length × width × height (1 m × 0.5 m × 0.4 m). The space of the cage is enough for five rats. They were provided by the Laboratory Animal Centre, Xuzhou Medical College, Xuzhou, China. The rats were housed at an ambient temperature of 25 ± 2 °C and relative humidity 45–55% with 12 h light/dark cycles. There are five rats per cage. They had free access to standard rodent pelleted diet and water ad libitum. The food was withdrawn 12–18 h before the surgical procedure. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Xuzhou Medical College.

2.2. Surgical procedure and luteolin treatments

Surgery to induced chronic cerebral hypoperfusion was carried out as described (Liu et al., 2005) with 10% chloral hydrate (350 mg/kg, ip), and common carotid arteries were exposed bilaterally and carefully separated. In sequence, the bilateral common carotid arteries were doubly ligated with 4-0 type surgical silk whereas not ligated in sham-operated rats. During the surgery, body temperature was maintained at 37.5 ± 0.5 °C.

Four days after surgery, the hypoperfused rats were randomly divided into 5 groups: sham; 2-VO animals; 2-VO animals + 50 mg/kg per day of luteolin (provided by Chengdu XiYa Chemical Technology Co., Ltd. China; suspended in 0.5% carboxymethylcellulose solution); 2-VO animals + 100 mg/kg per day of luteolin; and 2-VO animals + 200 mg/kg per day of luteolin. Daily oral administration of luteolin started on week 5 post-surgery and lasted for 8 weeks. The sham animals and 2-VO animals were administered equal-volume of 0.5% carboxymethylcellulose without luteolin. During the last 6 days of drug administration, spatial learning and memory were assessed in all rats. During the behavioral test, drugs were administered 40 min before the water maze training.

2.3. Morris water maze task

Animals were tested in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006). The apparatus consisted of a circular water tank (150 cm in diameter and 50 cm high), a platform (17 cm in diameter and 31 cm high) invisible (Prepared Chinese ink was added in the water) to the rats, and a computer program called WaterMaze's tracking system for automatically tracking and analyzing the path of an animal swimming in a large pool of water. The tank was filled with water maintained at approximately 25 ± 0.5 °C at a height of 32 cm, and the platform was inside and located in the middle of a certain quadrant about 22 cm distance from the tank wall. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The water maze task was carried out for 5 consecutive days. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. For each trail, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 90 s, it was gently placed on the platform and allowed to remain there for the same amount of time (Kuhad and Chopra, 2007, 2008). The time to reach the platform (latency in seconds) was measured.

On the sixth day, a probe trial was performed (Tuzcu and Baydas, 2006) wherein the extent of memory consolidation was assessed. The time spent in the target quadrant indicates the degree of memory consolidation that has taken place after learning. In the probe trial, the hidden platform was removed from the pool, and the rat was placed into the pool as in the training trial. The percentage of time spent in the former platform quadrant was taken as a measure of spatial memory retention.

2.4. Biochemical analysis

Following the behavioral testing, rats were decapitated under anesthesia. After the rats were sacrificed, the brain cortex and hippocampus were removed on ice. The tissues were rapidly frozen and stored at −80 °C until assays were performed. Brain tissues were homogenized and the supernatant was used to determine the activity of superoxide dismutase (SOD), and the level of malondialdehyde (MDA) and reduced glutathione (GSH) by spectrophotometry, in accordance with the protocol provided with the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China). MDA, the degradation production of peroxidized lipids, reacted with thiobarbituric acid to form a pink chromogen that was assayed by the method of Ohkawa et al. (1979). SOD activity represents the capability of cells to clear free radicals, the activity was assessed using the procedure described by Sun et al. (1988). GSH is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species (ROS) and the concentrations were determined by the procedures of Elmann (1959).