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Neurobiological and pharmacological validity of curcumin in ameliorating memory performance of senescence-accelerated mice

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ABSTRACT

The senescence-accelerated mouse prone 8 (SAMP8 mice) is known as a neurodegenerative model and may show age-related deficits of cognition. Curcumin, a major active component of spic turmeric, could increase the capacity of learning and memory in the aged rat. However, it is not known whether curcumin could improve cognitive deficits in SAMP8 mice. The present study was undertaken to evaluate the effect of curcumin on the learning and memory of SAMP8 mice and its possible mechanisms. Subjects were randomly divided into four groups: SAMR1 mice, SAMP8 mice and two SAMP8 mice groups treated, intragastrically, with curcumin at the dose of 20 and 50 mg/kg per day, respectively. After 25 days, spatial memory, superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, p-calcium/calmodulin-dependent kinase II (p-CaMKII) and p-N-methyl-D-aspartate receptor subunit 1 (p-NMDAR1) expression in the hippocampus of mice were examined by using the Morris water maze, biochemical analysis, immunohistochemistry and Western blot. Compared with SAMR1 mice, SAMP8 mice had longer escape latency, higher MDA content, lower SOD activity in the hippocampus, and lower intensity of p-CaMKII in the stratum lucidum of hippocampal CA3 and p-NMDAR1 expression in the hippocampal membrane fraction. Both 20 and 50 mg/kg curcumin administration significantly shortened the escape latencies and decreased the hippocampal MDA content in the SAMP8 mice. 50 mg/kg curcumin administration significantly ameliorated the hippocampal SOD activity, and increased the intensity of p-CaMKII in the stratum lucidum of hippocampal CA3 and p-NMDAR1 expression in the hippocampal membrane fraction of the SAMP8 mice. The present study demonstrated that curcumin treatment could attenuate cognitive deficits of SAMP8 mice in a dose-dependent manner by decreasing the oxidative stress and improving the expression of p-CaMKII and p-NMDAR1 in the hippocampus. Thus treatment with curcumin may have a potential therapeutic agent for aging-related cognitive dysfunctions.

1. Introduction

Aging is a risk factor for the deterioration of brain function whose main symptom is cognitive deficits (de la Torre, 2002). The senescence-accelerated mouse (SAM) initially obtained from AKR/J strain is thought as a neurodegenerative model (Takeda, 2009). In particular, SAMP8 mice are known as a model of senile dementia and may show age-related deficits of cognition (Nomura and Okuma, 1999). At an early stage and development of aging, the pathology of age-related disease was associated with oxidative stress (Barja, 2004). Oxidative stress induced the impairment of learning and memory, while antioxidants could improve the learning and memory and decrease oxidative stress in SAMP8 mice (Farr et al., 2003; Yasui et al., 2002). In some studies, age-related oxidative stress has been implicated in the down-regulation of expression of the protein substrates for memory, including the synapsins, synaptophysin and drebrin (Baldelli et al., 2007; Dun and Chilton, 2010; Reddy et al., 2005; Yamagata, 2003).

Evidence has indicated that cognitive deficits in SAMP8 mice seemed to be a consequence of dysfunction of some key proteins such as calcium/calmodulin-dependent kinase II (CaMKII) and N-methyl-D-aspartate receptor (NMDAR), which were essential for synaptic plasticity (Armbrecht et al., 1999; Hudmon and Schulman, 2002; Lisman et al., 2002). The increase of synaptic efficacy required the activation of NMDAR and CaMKII (Jourdain et al., 2002; Luscher et al., 2000; Segal et al., 2000). CaMKII and NMDAR are considered as molecular basis underlying learning and memory (Lau et al., 2004). Cheli et al. (2006) reported that NMDAR subunit 1 (NMDAR1), which was required for functional receptor formation, was knocked down in the hippocampus before the learning and memory in rats were impaired (Lau et al., 2004). Moreover, NMDAR stimulation could trigger the phosphorylation of CaMKII (Vaynman et al., 2007). As the high content of protein in the hippocampus and

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the main ingredient of post synaptic density (PSD), CaMKII is also considered as a molecular switch mediating the all-or-none potentiation of synapses (Chen et al., 2001).

Our previous study stated that *Rhizoma curcumae* oil could enhance the ability of learning and memory in rats exposed to chronic hypoxia (Sun et al., 2008). Its mechanism responsible for the improvement of cognitive function might be related to the effect of curcumin. Curcumin, a major component of *R. curcumae* oil or spice turmeric, is a yellow phenolic pigment isolated from the rhizomes of the plant *Curcuma longa* Linn. Turmeric has been used for the treatment of disease associated with injury and inflammation (Lodha and Bagga, 2000). Recent reports indicated that curcumin could increase the capacity of learning and memory by decreasing or inhibiting lipid peroxidation in the brain region of an aged rat (Bala et al., 2006; Pan et al., 2008). Curcumin could also ameliorate the learning and memory in the SAMP8 mice (Sun et al., 2011).

The present study is to elucidate the potential mechanism underlying the curcumin-induced improvement of learning and memory in the SAMP8 mice by observing the changes in the markers of oxidative stress and its scavengers such as malondialdehyde (MDA) and superoxide dismutase (SOD), and synaptic protein including p-CaMKII and p-NMDAR1 in the hippocampus as well.

2. Materials and methods

2.1. Animals

Experiments were performed using 6-month-old male SAMP8 mice and senescence-accelerated-resistant strain mice (SAMR1 mice) which were obtained from Tianjin University of Traditional Chinese Medicine [qualified number: SCXK (Tianjin) 2008-0001, Tianjin, China]. SAMR1 mice with normal aging process were selected as the control (Miyamoto et al., 1986). Experimental operation was consented by the Institutional Animal Ethic and Use Committees of Wenzhou Medical College (Wenzhou, Zhejiang Province, China). Mice were kept under controlled temperature (25 ± 1 °C), humidity (65 ± 5%) and a 12 h light/12 h dark cycle (07:30 to 19:30) with continuous access to food and water. Curcumin was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in corn oil before administration. Mice were randomly divided into 4 groups (22 animals in each): SAMR1 mice, SAMP8 mice and SAMP8 mice treated with curcumin, intragastrically (i.g.), at 20 and 50 mg/kg body weight, respectively. Both SAMR1 mice and SAMP8 mice were fed the same amount of corn oil. All four groups of mice were administrated once daily for 25 consecutive days. On the second day after the last administration, 10 mice from each group were subjected to behavioral tests.

2.2. Evaluation of spatial memory by Morris water maze

The Morris water maze consisted of a circular pool (1.8 m in diameter and 0.6 m in height), which was filled to a depth of 35 cm with water (22–24 °C) made opaque with milk. The maze was divided into 4 quadrants with one circular escape platform (10 cm in diameter) positioned at the center of arbitrary quadrant and submerged 1 cm below the water surface. Extra cues surrounding the maze were placed at specific locations and were visible to the mice. The navigation of each mouse was tracked by a video camera suspended above the maze and interfaced with a video tracking system (SMART® system, Spain).

Before the spatial working memory task, mice were given an acclimation session in the absence of the platform until they obtained a similar level of performance. When released, the mice did not swim around the side of the pool but search to go to the center of the pool within 60 s (>95%). On the following day, mice were trained in four trials per day task at 15 min intervals for 3 consecutive days. In each training trial, the mice were placed in the pool from start point. The time for mice to find the platform was specifically recorded if it was within 60 s (escape latency). If a mouse did not locate the platform within 60 s, that mouse would be manually guided to it, and the time of 60 s would be recorded. Performances of mice were assessed by the mean escape latencies. The daily trial data of each mouse was averaged and expressed as a block of four trials. Because the motor deficits might affect their performance in Morris water maze, swimming speeds in mice were also analyzed.

2.3. The biochemical analysis of MDA and SOD in mice hippocampus

On the second day after the last administration, another 6 mice in each group were sacrificed by decapitation. The brains were immediately taken out and divided into right and left halves. The right side of the hippocampus of each mouse was rapidly dissected out on an ice-cold plate. The tissue samples were homogenized in 0.9% normal saline (NS) and the 10% homogenate was performed to measure MDA-reactive products as described by Todorova et al. (2005) using the method of thiobarbituric acid reactive substance (TBARS). The amount of MDA was quantified spectrophotometrically at 532 nm by reaction with TBA and expressed as nanomoles of MDA/g of wet hippocampus using molar extinction coefficient of the chromophore (1.56×10^{-5} M/cm). Superoxide dismutase activity was measured by commercial kits purchased from Cayman Chemical Company (Ann Arbor, Michigan). One unit of SOD was defined as the amount of enzyme required for producing 50% dismutation of the superoxide radical and 50% conversion of the hydrogen peroxide, respectively. Absorbance was measured for 5 min at 420 nm and the final SOD activity was expressed as units of enzymatic activity per mg protein contained in the samples (U/mg protein). MDA and SOD assays were repeated three times for each sample.

2.4. Preparation of hippocampal membrane fraction and Western blot for p-NMDAR1

The left side of the hippocampus from 6 mice in each group that have been motioned was homogenized in 0.9% NS, and then centrifuged at 3000 rpm for 5–10 min. The residua were transferred to lysis buffer consisting of 10 mg/ml PMSF, 50 mM Tris HCl (pH 7.4) and 0.5 M EDTA. The lysate sample was then centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant (cytoplasmic fraction) was placed into the buffer including 10 mg/ml PMSF, 50 mM Tris HCl (pH 7.4) and 30% Triton X-100 and then centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant (total membrane fraction) was transferred to a new chilled tube and the integral membrane protein concentration was determined by a BCA™ protein assay kit (Thermo Scientific, USA). After denaturing at 95 °C for 3 min, each protein sample (15 μl) was loaded to the bottom of the each well, electrophoresed on SDS-PAGE, and then electro-blotted onto a PVDF membrane (Bio-Rad Laboratory, USA). The membranes were incubated in blocking buffer consisting of 5% non-fat dry milk in 0.01 M PBS for 1 h at room temperature and then treated with anti-p-NMDAR1 (Ser896) rabbit polyclonal antibody (1:200 dilution) and anti-GADPH mouse monoclonal antibody (1:1000 dilution) (Santa Cruz Biotechnology, USA) for 24 h at 4 °C, respectively. After rinsing, the membranes were incubated with anti-rabbit or anti-mouse secondary antibodies conjugated with horseradish peroxidase (Beijing Zhongshan Biotechnology Co., China). The intensity of immunoreactive bands was detected by an enhanced chemiluminescence system (Beijing Zhongshan Biotechnology Co., China) and photographed on the gel image analysis system (Bio-Rad, USA). The signal intensity for p-NMDAR1 from each band was normalized to the corresponding GADPH expression and analyzed by ImageJ program (NIH, version 1.39×).