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Effects of safflower yellow on beta-amyloid deposition and activation of astrocytes in the brain of APP/PS1 transgenic mice

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ABSTRACT

Safflower yellow (SY), one of traditional Chinese medicine extracted from safflower, has been shown to have neuroprotective effects on animal models of vascular dementia and Alzheimer's diseases (AD), by inhibiting oxidative injury, neuronal apoptosis and tau hyperphosphorylation. In this study, we investigated whether safflower yellow (SY) can improve cognitive function, decrease Amyloid β (Aβ) accumulation and overactivation of astrocytes in AD mouse model. We found that SY treatment significantly ameliorated the learning and memory deficits of APP/PS1 mice. By hematoxylin-eosin staining, we found that the neuronal loss and death in APP/PS1 mice was decreased by SY treatment. Immunohistochemical staining showed that SY treatment dramatically down-regulated $A\beta_{1\text{-}42}$ deposition and glial fibrillary acidic protein (GFAP) level in APP/PS1 mice. Biochemical analysis also showed that SY treatment reduced soluble and insoluble $A\beta_{1\text{-}42}$ level in the cortex and soluble $A\beta_{1\text{-}42}$ level in the hippocampus of APP/PS1 mice. Moreover, we found that SY treatment decreased the expression of proteins related to generation of Aβ, and markedly increased expression of enzymes associated with clearance of Aβ in the brain of APP/PS1 mice. These results indicate that the SY can serve as a promising therapeutic approach for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a multifactorial and progressive neurodegenerative disease. The main hallmarks of Alzheimer's disease are Amyloid β (Aβ) plaques and neurofibrillary tangles accompanied by evidences of neuroinflammation [1]. Increasing evidences demonstrated that the deposition of Aβ peptides in the brain plays a key role in the development of AD. It causes malfunctions of the synaptic transmission, oxidative stress and the final death of neurons. Moreover, further researches on the disease have also reported that inflammatory responses have an impelling action on the neuropathology of AD [2,3]. A combination of pathological factors results in an imbalance between Aβ production and clearance, leads to formation of Aβ oligomers and finally accumulates as senile plaques in the brain [4]. Aβ oligomers, including $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, have been shown to have neurotoxic effects in many studies. Meanwhile, they also have the ability to activate microglia and astrocytes, and promote the secretion of various inflammatory cytokines. The activated microglia and astrocytes are always found to surround the senile plaques. During the pathogenesis of AD, the relationship between Aβ and activated microglia/astrocytes is both close and contradictory [5,6]. On the one hand, the astrocytes and microglia activated by Aβ will release a series of cytokines and inflammatory factors, such as inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1 β), and tumor necrosis factor alpha (TNF-α) [7,8]. These cytokines participate in the central lesion and ultimately cell death. On the other hand, the activated microglia will internalize and degrade toxic Aβ peptides in vitro, and participate in the clearance of Aβ from the brain via phagocytosis [9,10].

Safflower yellow (SY) is a main active chemical ingredient isolated from traditional Chinese medicine *Carthamus tinctorius* L. The main components of SY are hydroxyl safflower yellow A (HSYA) and safflower yellow B (HSYB), which have been widely used for treatment of cerebrovascular, cardiovascular diseases, and following traumas. These studies suggest that SY have significant anti-inflammatory and antioxidation effect [11,12]. Our previous study has shown that SY treatment can improve the learning and memory ability, decrease tau

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neuropathology, and enhance the antioxidant capacity of brain in an AD rat model induced by $A\beta_{1-42}$ [13]. However, it still remains unclear whether SY treatment has effects on the metabolism of Aβ in APP/PS1 transgenic mice. In this study, we investigated the effect of SY treatment on cognitive function, Aβ accumulation and astrocyte activation in AD mouse model. Our results suggest that SY treatment can significantly ameliorate the pathogenesis of AD.

2. Materials and methods

2.1. Animals

Forty male 9-month-old APP/PS1 double-transgenic mice with a C57BL/6JNju background and ten male wide type 9-month-old C57BL/6JNju mice and were purchased from Nanjing Biomedical Research Institute of Nanjing University [strain name, APP/PSN (B6) –Tg (APPswe, PSEN1dE9) 85Dbo; stock number, D000268]. All mice weighing 25–30 g can arbitrarily access to water and are fed with standard rodent food in a 22 ± 2 °C environment under a 12 h light/12 hr dark cycle (8:00 am–8:00 pm.) until they reached the desired age (9– 10 months). The genotypes of the transgenic mice were determined by PCR analysis of genomic DNA from tail biopsy samples. We selected APP/PS1 mouse that have high levels of Aβ, as well as senile plaque formation in the brain.

2.2. Drug treatment

Safflower yellow (Yunnan Tonghai Yangshi Biotechnology Co, China) was prepared at 4 °C in distilled water at concentrations to provide the desired doses of 10, 30 and 100 mg/kg and protected from light. 40 APP/PS1 mice were randomly divided into five groups: saline treated group (n = 8), SY treatment groups (10 mg/kg concentrations, n = 8, 30 mg/kg concentrations, n = 8, 100 mg/kg concentrations, n = 8), positive drug galanthamine hydrobromide group (3 mg/kg concentrations, n = 8). Meanwhile, wild-type mice were selected into normal control group (n = 10). The galanthamine hydrobromide solution and different concentrations of SY solution were administered to the APP/PS1 mice by intragastric administration (0.01 ml/g body weight) daily for 90 days. Whereas, the saline treated group and wide type group were received the same volume of normal saline.

2.3. Morris water maze test

The Morris water maze test was performed as previously described with some modifications. During six days all mice were evaluated using the same program and equipment (Chinese Academy of Medical Sciences & Peking Union Medical College, DMS-2). The circular pool (height: 40 cm, diameter: 100 cm) was filled with water that had been dyed by adding milk powder and maintained at 18–23 °C. It was divided into four quadrants I, II, III and IV. An escape platform (height: 10 cm, diameter: 15 cm) was positioned in the center of the III quadrant and hidden 1 cm below the water surface. In these trials, 40 APP/PS1 mice and 10 wild-type mice were released into the water facing the wall of the pool from semi randomly in turn of I, II, III and IV. At the same time, we timed for its latency to reach the escape platform for a maximum time of 1 min. If the platform was not found within 1 min, the mice were directed to the platform for 10 s and the latency was recorded as 60 s. They accomplished four training trials per day in a total of five days. On the sixth day, the space probe test was performed. During the test, the platform was removed entirely and mice were allowed to swim in 60s from the opposite and adjacent quadrants of the original platform in order to find about the position of the platform. The place navigation and space exploration tests can serve as a reflection of the learning and memory of the goal location. The time across the platform and percentage of time in the platform quadrant were measured by the automatic video analysis system.

2.4. Step-down test

A single trial step-down passive avoidance task was used for memory assessment in mice. The step down device was composed of a (60 × 33 × 11) cm big box with a floor that made of parallel stainless steel bars and a wooden platform was placed on the center of it. An alternating electric current transferred an electric shocker (AC, 50 Hz) to the grid floor. During the training session, the first day 40 APP/PS1 mice and 10 wild-type mice were placed in a reaction chamber adapted for 3 min and they would hop wooden platform after received an electrical shock under normal circumstances. We recorded the time to jump on the platform and error times of jump off it within 5 min which developed from a variety of continuous electric stimulation. Mice were placed onto the platform inside a cylinder for 5 min which the shocking experience occurred.

2.5. Tissue preparation

After behavioral tests, all mice were anesthetized by chloral hydrate, quickly perfused with PBS, and half of their brain were fixed with 4% paraformaldehyde at 4 °C for 48 h, the other half of the brain tissues were directly separated into cortical and hippocampal regions, and then immediately stored at −80 °C. We carried out HE staining and immunohistochemical staining on the half of 40 APP/PS1 and 10 wild-type mice brain. The other half of the hippocampus and cortex were used for the Western blot and Elisa methods.

2.6. HE staining

Forty APP/PS1 and ten wild-type mice have been used in the HE staining experiment and immunohistochemistry. The left half of brains fixed in 4% paraformaldehyde for 48 h at 4 °C after hearts perfusion with cold PBS. Brains were directly dissected and coronal brain sections of 3 μm thickness were prepared using a microtome (Leica RM2245; Leica Microsystems Nussloch GmbH, Germany). Then the sections were steeped into 95% alcohol for 40 min after applying labels to them. Next the tissue slices were immersed in absolute ethyl alcohol for 1 h followed by xylene for 30 min. The tissues were infiltrated into liquid paraffin at 70 °C for 90 min and finally embedded in paraffin blocks. Then sections were heated at 70 °C for 1 h and incubated with xylene for 5 min for 3 times. Paraffin sections were rehydrated by a graded series of alcohols (absolute alcohol to 95%, 80%, 70%) for 5 min each grade. Then the slices were counterstained in Harris-modified hematoxylin solution for 5 min and rinsed with distilled water for 30 s for 5 times. Subsequently, we dipped them in hydrochloric acid alcohol solution for 30 s to decolor, and rinsed with distilled water. The stained sections were observed under the microscope (Optec instrument Co., Ltd, Chongqing, China) at 100× magnification. Finally, sections were stained in eosin-Phloxine solution for 2 min then followed by static washing for 3 times, dehydrated with ethyl alcohol (70%, 80%, 90%) for three seconds and absolute alcohol for 5 min for 2 times, cleared in xylene for 5 min for twice. Each slide was covered with a drop of permount TM mounting medium relied on glass rods and dried overnight. Pictures were taken by Olympus CX31 microscope (Olympus, Japan).

2.7. Immunohistochemistry

Sections were immersed in xylene for 5 min for 3 times, and rehydrated in progressively dilute ethanol solutions (absolute alcohol to 95%, 80%, 70%) for 5 min and washed in distilled water for 5 min for 3 times. Antigen retrieval was achieved with a microwave in citrate buffer (10 mM, pH 6.0) for 3 min at a high temperature and 10 min at a low temperature. In order to abolish endogenous peroxidase activity, the slices were treated with 3% H_2O_2 in. 1 M phosphate buffer solution for 10 min at room temperature. The sections were incubated with primary anti-rabbit polyclonal glial fibrillary acidic protein antibody

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