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Acetylshikonin from Zicao attenuates cognitive impairment and hippocampus senescence in D-galactose-induced aging mouse model via upregulating the expression of SIRT1

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ABSTRACT

Zicao acts as a pleiotropic medicine in various diseases due to its particular pharmacological properties, including anti-inflammatory, anti-tumor, anti-oxidative, and wound healing effects. However, few studies have focused on the function in neurodegenerative diseases of Zicao. In this study, we investigated the neuroprotective effect of Acetylshikonin (AS) from Zicao on the hippocampus of the D-galactose (D-gal)-induced sub-acute aging mouse model of Alzheimer's disease (AD). The aging model was established in male Kunming mice by subcutaneous injection of D-gal (150 mg/kg/d) for 60 days, and the mice were given AS (270, 540 and 1080 mg/kg/d) or distilled water intragastrically for 30 days after 30 days of D-gal injection. The behavioral results test by Morris Water Maze (MWM) revealed that chronic AS treatment alleviated D-gal-induced learning and memory deficits compared with the D-gal-treated mice. In addition, AS also ameliorated the oxidative stress and neuroinflammation induced by D-gal through decreasing the level of interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), malondialdehyde (MDA) and enhancing the activity of the antioxidant enzymes superoxide dismutase (SOD). Moreover, western blot results showed that AS can up-regulate the expression of Sirtuin 1 (SIRT1) and inhibit D-gal-induced activation of p53/p21 signaling pathway in the hippocampus of mice. These results suggest that AS can execute the prevention and treatment of D-gal-induced brain aging by SIRT1/P53/P21 pathway.

1. Introduction

Aging is one of the greatest risk factors for the Alzheimer's disease (AD), the most prevalent aging-associated neurodegenerative diseases characterized by progressive memory loss and cognitive dysfunction in the elderly people over 65 years old (Weuve, 2016), and the underlying molecular mechanisms for AD are not fully understood. Numerous studies have shown key roles of oxidative stress, neuroinflammation and neuronal apoptosis in the pathogenesis of AD (Heneka et al., 2015). D-Galactose (D-gal), a reducing sugar normally present in the body, can be oxidized into aldehydes and hydrogen peroxide by galactose oxidase at the high levels. Evidences have shown that abnormal D-gal metabolism caused aging-related changes, including progressive deterioration of learning and memory capacity, and increased the production of reactive oxygen species (ROS) and lowered activities of the antioxidant enzymes (Yang et al., 2014). Therefore, chronic injection of D-gal has been regarded and widely used as an ideal model to study the possible mechanisms of aging-related neurodegenerative diseases, especially for AD.

SIRT1, the silent mating type information regulation 2 proteins 1 of class III histone deacetylases, is associated with health span and longevity and plays crucial roles in neuronal plasticity, cognitive functions, as well as protection against aging-related neuronal degeneration and cognitive decline (Ng et al., 2015). It was investigated that SIRT1 has an inherent role in learning and memory and its over-expression in animal models suppressed AD-associated pathology, which implied SIRT1 as a therapeutic target for AD (Chang et al., 2015). What's more, Kim et al. demonstrated that resveratrol, a SIRT1-activating molecule, protected against neurodegeneration in models for Alzheimer's disease (Kim et al., 2007). P53, a tumor suppressor, can be activated by numerous stressors to induce apoptosis, cell cycle arrest, or senescence and deacetylated by SIRT1 and also plays an essential part in aging (Rodier et al., 2007). P21, also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1, is capable of inhibiting all cyclin/CDK complexes and represents a major target of p53 activity and thus is associated with linking DNA damage to cell cycle arrest (Karimian et al., 2016). Considering all the findings above, we hypothesized that SIRT1, P53 and P21 emerged as important indicators in the sub-acute aging model of mice induced by D-gal.

Zicao, an herbal belongs to Boraginaceae family, has been

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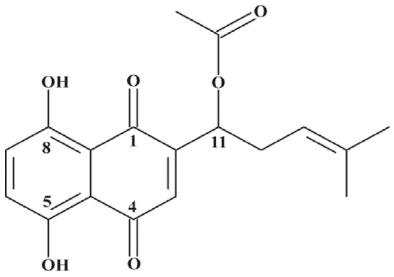

Fig. 1. Chemical structures of Acetylshikonin.

commonly used in clinics as a traditional Chinese medicine for centuries in China, and its primary active ingredients are naphthoquinone derivatives containing acetylshikonin (AS, Fig. 1), isovalerylalkannin, shikonin, β-hydroxyisovaleryshikonin, deoxyshikonin, and β,β-dimethylacrylshikonin (Chen et al., 2002). Its derivatives have revealed various pharmacological activities including anti-inflammatory, anticancer, antimicrobial and wound healing effects. The research results of our group indicated that β, β-dimethylacrylshikonin promoted the angiogenesis of human umbilical vein endothelial cells and accelerated the wound healing process in diabetic rats (Zeng and Zhu, 2014). Our studies also demonstrated that AS from Zicao prevented obesity and hepatic steatosis in db/db mice and inhibited lipid accumulation and induced lipolysis in rats on a high-fat diet (Su et al., 2016). Moreover, our previous research found that AS from Zicao exerted antifertility effects at high dose, which providing credence to the use of Zicao as a valuable and secure source of modern drugs (He et al., 2016).

Acetylshikonin, one of the main active components of Zicao, is a highly lipophilic naphtoquinone compound which could readily penetrate the Blood-Brain-Barrier. Our previous findings suggest that AS can act on the hypothalamic-pituitary-gonadal axis and decrease the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by affecting exocytosis process of gonadotropic hormone (GTH), implying that AS can act through the blood-brain barrier in the Central Nervous System. Furthermore, Wang et al. have indicated that AS and its derivatives exhibited potent anti-apoptotic activity in the neuronal cells and attenuated H_2O_2-induced oxidative stress (Wang et al., 2013). Moreover, we found that the mental state of mice or rats with diabetes or obesity administrated by AS would be greatly improved in the previous research, suggesting that AS would become a beneficial and valuable agent in the neurodegenerative diseases. Therefore, in this study we aim to investigate whether AS could exert neuroprotective effects and further explore the potential molecular mechanisms. The D-gal-induced sub-acute aging model of mice were adopted, the cognitive ability of mice were evaluated by Morris Water Maze (MWM), inflammation and oxidative stress-related indicators were determined by the corresponding kits and the possible related proteins, including SIRT1, P53, Acetyl-p53 (Ac-p53) and P21, were measured by western blot.

2. Materials and methods

2.1. Reagents and antibodies

Zicao were acquired from Xinjiang, China (AS and its extract are both derived from the Zicao at purities of 98.4% and > 80%, respectively). D-Gal was ordered from Sigma-Aldrich Corporation (St. Louis, MO, USA). IL-1β ELISA Kit and TNF-α ELISA Kit were purchased from Wuhan Boster Bio-engineering Co, Ltd. (Wuhan, China). MDA and SOD kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA kit and SA-β-gal Staining kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Primary antibodies including SIRT1, P53, Ac-p53, P21 and GAPDH and all secondary antibodies for western blot were purchased from Cell Signaling Technology (Danvers, MA, USA). Reagents were obtained from Sigma-Aldrich unless otherwise specified.

2.2. Animal grouping and treatments

Eight weeks old male Kunming mice were purchased from Animal center of Sun Yat-sen University and housed in a temperature and light-controlled room with free access to water and food. All experiments were performed in accordance with institutional and national guidelines and regulations and were approved by the Animal Research Center of Sun Yat-sen University. Totally 40 mice were randomly divided into 5 groups with 8 mice in each group: (1) control, (2) D-gal-administration (150 mg/kg), (3) D-gal-administration plus AS treatment (270 mg/kg), (4) D-gal-administration plus AS treatment (540 mg/kg), (5) D-gal-administration plus AS treatment (1080 mg/kg). After adapting to new environment for 1 week, the mice from D-gal-administration group and D-gal-administration plus AS treatment group were injected subcutaneously daily with D-gal (150 mg/kg) dissolved in saline at 10:30am for 8 consecutive weeks, while the control group were administered with same volume saline. In the D-gal-administration plus AS treatment group, AS extract (270, 540 and 1080 mg/kg) was administrated intragastrically daily at 8:30am for 30 days after 30 days of D-gal injection. The mice from control group and D-gal-administration group were given the same volume of distilled water orally, respectively. Behavioral testing was carried out after 8 weeks of drugs treatment.

2.3. Behavioral testing

Morris Water Maze test was widely applied to evaluate spatial learning and memory capacity of the D-gal-induced aging mice and we, in this research, performed MWM as described previously with minor modification (Morris, 1984). The experimental equipment is consisted of a circular water tank (120 cm in diameter,40 cm in height), containing water (24 ± 1 °C) to a depth of 25 cm, which was rendered opaque by adding milk powder. A platform (8 cm in diameter) was submerged 1 cm under water in the midpoint of one of four identical quadrants. The MWM task lasted 6 days, including 5-days place navigation training and a probe test on day 6. In the 5-days oriented navigation trials, each mouse received training per day using a single hidden platform in one quadrant with three quadrants of rotational starting and the latencies to find the hidden platform was recorded. Mice were allowed a maximum of 120 s to find the platform and those failed within 120 s were gently guided to the platform and allowed to stay on the platform for 15 s. On the sixth day, probe test was performed for the evaluation of memory consolidation. Each mouse was allowed to swim freely for 120 s with the platform removed in the probe trial and the numbers of target crossings over the previous location of the target platform were also recorded.

2.4. Tissue collection and sample preparation

After behavioral analysis, one half of the mice in each group were sacrificed to obtain the serum and brain tissues, while the rest were killed for histological analysis. Blood was collected from the abdominal vena cava and centrifuged at 3000 rpm for 15 min at room temperature. The brains were immediately removed and the hippocampus tissues were separated carefully, and stored at −80 °C for the biochemical analysis. Another 4 mice in each group were anesthetized and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde and the brains were immersed in 4% paraformaldehyde at 4 °C overnight. Then the brains were respectively followed by dehydration in 5%,10% and 20% sucrose at 4 °C. 20 um coronal sections were cut by cryostat microtome and fixed on microslide for Senescence SA-β-galactosidase (SA-β-gal) staining after brains were frozen in O.C.T compound.