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Paeonol attenuates neurotoxicity and ameliorates cognitive impairment induced by D-galactose in ICR mice

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ABSTRACT

In the present study, we examined the supplementation of paeonol extracted from *Moutan cortex* of *Paeonia suffruticosa* Andrews (MC) or the root of *Paeonia lactiflora* Pall (PL) on reducing oxidative stress, cognitive impairment and neurotoxicity in D-galactose (D-gal)-induced aging mice. The ICR mice were subcutaneously injected with D-gal (50 mg/(kg day)) for 60 days and administered with paeonol (50, 100 mg/(kg day)) simultaneously. The results showed that paeonol significantly improved the learning and memory ability in Morris water maze test and step-down passive avoidance test in D-gal-treated mice. Further investigation showed that the effect of paeonol on improvement of cognitive deficit was related to its ability to inhibit the biochemical changes in brains of D-gal-treated mice. Paeonol increased acetylcholine (Ach) and glutathione (GSH) levels, restored superoxide dismutase (SOD) and Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase) activities, but decreased cholinesterase AChe activity and malondialdehyde (MDA) level in D-gal-treated mice. Furthermore, paeonol ameliorated neuronal damage in both hippocampus and temporal cortex in D-gal-treated mice. These results suggest that paeonol possesses anti-aging efficacy and may have potential in treatment of neurodegenerative diseases.

1. Introduction

Aging is an important factor leading to the dysfunction of the normal cellular modulation, affecting various systems such as nervous system and immune system [1]. It is a causative factor of diverse chronic diseases, including cancer, cardiovascular diseases and neurodegenerative diseases. Anti-aging has already become a major public issue with the increasing elderly population in the world.

Abundant evidence has pointed to an important role of oxidative stress during the pathogenesis of brain aging, age-associated or neurodegenerative diseases [2–5]. Indeed, with age increasing, accumulation of oxidant damaged cellular macromolecules, such as DNA, proteins and lipids of cell membranes [6,7]. Brain, with high oxygen demand, high level of unsaturated lipids, and relatively deficient in anti-oxidative defense mechanism, is the most susceptible organ to oxidative damage. Thus, antioxidant therapy may be an important avenue for managing neurodegenerative diseases.

D-galactose (D-gal) can be metabolized at normal concentration. But when at high levels, it can be converted into aldose and hydroperoxide under the catalysis of galactose oxidase, resulting in the generation of a superoxide anion and oxygen-derived free radicals (OFR) [8,9], which impair macromolecules and cells. Studies with D-gal-lesioned rodents have shown that D-gal-induced oxidative stress initiates cell apoptosis and results in various changes including neurodegeneration. Reports demonstrated that mice continuously exposed to high dosage of D-gal showed an increase in levels of cleaved capase-3, apoptosis and karyopyknosis in hippocampal neurons and a decline in spatial learning and memory [10,11]. The D-gal-treated rats had a decreased density of synapse on the catecholaminergic region and a forebrain cholinergic neuronal loss was also reported [12]. In addition, long-term injection of D-gal in mice impaired neurogenesis in the dentate gyrus and induced newly formed neurons death in the granular cell layer [13]. The D-gal-lesioned rodents have been used for brain aging studies, as D-gal induced behavioral and neurochemical changes can mimic many characters of the natural brain aging process [8–11,13].

Moutan cortex of *Paeonia suffruticosa* Andrews (MC) and the root of *Paeonia lactiflora* Pall (PL), the two Chinese crude drugs, have been used widely in the treatment of various diseases in China for thousands of years, such as antipyretic and anti-inflammatory purposes. Paeonol

Abbreviations: Ach, acetylcholine; AChe, acetylcholine esterase; D-gal, D-galactose; DTNB, dithio-bis-nitrobenzoic acid; GPx, glutathione peroxidase; GSH, reduced glutathione; MC, *Moutan cortex* of *Paeonia suffruticosa* Andrews; MDA, malondialdehyde; Na^+, K^+-ATPase, Na^+, K^+-adenosine triphosphatase; PL, *Paeonia lactiflora* Pall; ROS, Reactive oxygen species; SOD, superoxide dismutase.

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(Fig. 1) is a main active compound isolated from MC or PL. In addition to the activities of antipyresis and anti-inflammation, paeonol has also been suggested to have properties of anti-platelet aggregation [14], scavenging free radicals and antioxidation [15]. Moreover, paeonol proved to have activity in decreasing Ca^{2+} influx by blocking L-type Ca^{2+} channels [16]. An increase in Ca^{2+} influx through L-type Ca^{2+} channels is considered to be a causative factor of neuronal dysfunctions underlying senile symptoms and Alzheimer's disease [17]. As we have known, no previous work has been done to investigate whether the paeonol has an effect against D-gal-induced brain aging and neurotoxicity in mice model.

Based on the previous findings aforementioned, we hypothesized that paeonol could exert efficacy in preventing aging of the brain in D-gal-lesioned mice model. In the present study, we investigated the actions of paeonol on cognitive decline of aging mice induced by D-gal, acetylcholine/acetylcholinesterase, Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase) activities and antioxidant/oxidant parameters in the brain. In addition, we also investigated the effect of paeonol on the pathological changes in both cerebral cortex and hippocampus of the brain in D-gal-treated mice.

2. Methods and materials

2.1. Reagents

Paeonol (purity >99%) was purchased from Xuancheng Baicao Plants Industry and Trade Co., Ltd. D-galactose and kits used for determination of SOD, MDA, Na^+, K^+-ATPase, glutathione reductase and AChe, were purchased from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China). Kits used for determination of D-galactose were purchased from Biosentec (France). Other reagents were AR grade.

2.2. Animals and treatments

Male ICR mice, 5-week-old, were housed in groups of three to four per cage at a temperature of 22 °C±2 °C with a 12 h light–dark cycle (light on 7 a.m.–7 p.m.), relative humidity 50–70%, and had free access to the normal food (The experimental animal breeding center of China Pharmaceutical University) and water. The animals were cared in compliance with the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation and were approved by the Science and Technology Department of Jiangsu Province. After adaptation for a week, mice were randomly divided into 5 groups (15 in each group). Groups 1–4 of mice received daily subcutaneous injection of D-gal at a dose of 50 mg/(kg day) for 60 days. Meanwhile, groups 1–2 of mice received paeonol of 50, 100 mg/(kg day) in distilled water containing 0.1% Tween 80 by oral gavage, respectively, for 60 days, and group 3 of mice received vitamin E of 100 mg/(kg day) in distilled water containing 0.1% Tween 80 orally for 60 days. Group 4 of D-gal-treated mice that served as model received distilled water containing 0.1% Tween 80 without paeonol. Group 5 that served as vehicle control received daily subcutaneous injection of saline (0.9% NaCl) and distilled water containing 0.1% Tween 80 without paeonol.

2.3. Morris water maze test

Learning and memory ability was detected by Morris water maze test [11]. In this test, mice were trained to find a platform (6 cm in diameter) hidden 1 cm below the water surface in a circular water tank (100 cm in diameter, 45 cm in height). Each mouse received four training sessions per day for 4 consecutive days. One day before the first trial, each mouse received 4 times of pre-training: mouse was put on the platform for 20 s, then given a 30 s free swim and placed on the platform where it was allowed to rest for another 20 s rest. For each trial, the mouse was placed in the water facing the pool wall at one of four starting quadrant point, and the time required for the mouse to find the hidden platform was recorded. A mouse that found the platform was allowed to stay on it for 20 s and then returned to its cage for 40 s inter-trial interval. If the mouse did not find the platform within 60 s, it would be placed on it for 20 s, and the escape latency (finding the submerged platform) was recorded as 60 s. After the 4th training trail, the platform was removed and each mouse was allowed to swim freely for 60 s as the probe test. The time that mice spent in the target quadrant (where the platform was once hidden) was measured.

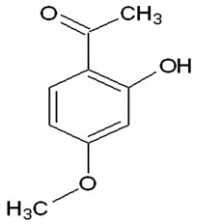

Fig. 1. Molecular structure of paeonol.

2.4. Step-down type passive avoidance test

The apparatus consisted of a plastic box divided into six equal cabins (20 cm×20 cm×25 cm high) with grid floor made of stainless steel bar spaced 1 cm apart (manufactured by Institute of Materia Medica, Academy of Medical Science, China). The rubber column platform (4 cm in diameter) was fixed in one corner of the cabins. Electric shocks (40 mV) were delivered to the grid floor with an isolated pulse stimulator. At the beginning of training, the animal was placed in the box to adapt for 3 min. Next, electric currents were delivered and the mice would jump onto the platform to avoid the electric stroke, and the electric currents were maintained for 5 min. After a 24 h interval, the mice were again placed on the platform, and the latency to step down on the grid with all four paws for the first time and the number of errors subjected to shocks within 5 min were measured as learning performances.

2.5. Preparation of tissue samples

Mice were decapitated 60 min after the behavioral tests. The cerebrum was separated on ice, longitudinally bisected along the axes. The left cerebral hemisphere was homogenized with ice-cold saline containing protease inhibitor cocktail (Sigma-Aldrich, MO, USA) to be 10% (w/v) homogenate.

2.6. Collection of brain slice

For pathological studies, following pre-fixed in 10% methanal for 24 h, the right cerebral hemisphere was postfixed in 70% ethanol for at least 12 h. After dehydrated, the brain was embedded in paraffin blocks. Four coronal sections of 10 μm at −4.0 mm posterior from bregma were stained with hematoxylin–eosin.

2.7. Assay of D-gal levels in plasma and brain

The D-gal levels in plasma and brain were measured according to the method of Hoffman [18]. Briefly, supernatant of homogenate or serum (proteins have been eliminated by perchloric acid) 100 μl was mixed with 200 μl of PBS (pH 10.0) followed by addition of PBS (pH 8.6) containing 1.7 mg of oxidized form of nicotinamide-adenine dinucleotide (NAD). Subsequently, 1950 μl of bidistilled water was added in the mixture. Then, 50 μl of D-galactose dehydrogenase (containing 1.2 U) was added and mixed sufficiently. The absorbance at 340 nm was monitored. D-gal levels were expressed as mmol/l or μmol/g wet weight.