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Research report

Beneficial effects of cornel iridoid glycoside on behavioral impairment and senescence status in SAMP8 mice at different ages

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HIGHLIGHTS

- CIG could not only treat early AD but also possess beneficial effects on moderate to severe AD.
- CIG has the potential effect to ameliorate the daily living qualities and the lifespan of AD patients.
- The pharmacological mechanism of CIG related to anti-hyperphosphorylation of tau in SAMP8 mice, which benefits to AD treatment.

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ABSTRACT

The aim of the present study was to investigate the effects of cornel iridoid glycoside (CIG) on behavioral changes and senescent status in senescence-accelerated mouse-prone 8 (SAMP8) mice at different ages (6, 10, and 14 months old). The learning and memory ability, the motor function and the aging conditions of SAMP8 mice were evaluated after CIG treatment in this study. Results showed that intragastrical administration of CIG (100 and 200 mg/kg) for two months obviously improved the impaired cognitive ability of SAMP8 mice at the age of 6 months and 10 months, respectively. The treatment with CIG significantly increased the motor function of SAMP8 mice at 10 months and 14 months of age, respectively. CIG also evidently decreased the high grading score of senescence and increased the low surviving rate of SAMP8 mice at the age of 14 months. In addition, CIG treatment inhibited tau hyperphosphorylation in the hippocampus and striatum of SAMP8 mice at different ages. Together, these results indicate that CIG represent a potentially useful treatment for ameliorating the impaired cognitive ability, the motor dysfunction, aging conditions and hyperphosphorylation of tau in aging and age-related neurodegenerative diseases, such as Alzheimer's disease.

1. Introduction

Aging is associated with the deterioration of memory and motor abilities and with an increased incidence of neurodegenerative disorders, such as Alzheimer's disease (AD) [1]. Senescence-accelerated mouse (SAM) is a murine model of accelerated aging, spontaneously developed from breeding pairs of the AKR/J series at Kyoto University [2]. Among the stains, senescence-accelerated mouse-resistant 1 (SAMR1) serves as a control exhibiting normal aging phenotype [3], while senescence-accelerated mouse-prone 8 (SAMP8) is one of the strains and widely used as aging-related neurodegeneration animal model [4]. SAMP8 displays irreversible advancing senescence and share similar characteristics with aged humans and AD patients, such as a shortened lifespan, obvious age-related deteriorative cognition [5–7], physical impairments [7,8], hyperphosphorylation of tau [9], and various pathological features of age-associated neurodegeneration, [4,10,11].

Cornel iridoid glycoside (CIG) is the active ingredient extracted from *Cornus officinalis Sieb. et Zucc*, a traditional Chinese herb widely used for treatment of dementia and other age-related diseases in China [12]. The main components of CIG are morroniside and loganin. According to a pharmacokinetic study conducted by Chen, et al. [13], both morroniside and loganin are absorbed into blood after intragastrical administration. The HPLC study of tissue distribution in rats has shown that both morroniside and loganin cross the blood-brain barrier [14,15]. In our previous studies, we found that CIG inhibited inflammation and apoptosis in the brain of rats with focal cerebral ischemia [16], promoted neurogenesis and angiogenesis and improved neurological function after stroke

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in rats [17]. CIG also ameliorated memory ability and promoted neuronal survival in fimbria–fornix transected rats [18]. Our recent studies have shown that CIG attenuates tau hyperphosphorylation by inhibiting PP2A demethylation *in vitro* [19]; morroniside, a main component of CIG, induces PP2A activation and antagonizes tau hyperphosphorylation in a cellular model of neurodegeneration [20]. However, it remains unknown whether CIG has anti-aging effects on SAMP8 mice or not.

Since aging is the most important risk factor of AD [21], and SAMP8 mice exhibit age-related AD-like pathological phenotypes, we investigated the effects of CIG on behavioral changes, senescent status and tau phosphorylation in SAMP8 mice at different ages (6, 10, and 14 months old) in the present study.

2. Materials and methods

2.1. Drugs

Cornel iridoid glycoside (CIG) was extracted from the sarcocarp of *Cornus officinalis Sieb. et Zucc*, as described previously [17]. The *C. officinalis* was purchased from Tong-Ren-Tang Company, Beijing, China. The purity of CIG was 70% as determined by high-performance liquid chromatography, in which morroniside accountd for 67% and loganin 33%.

2.2. Animals

All mice were supplied by the Experimental Animal Centre of the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine. Animals were housed with free access to food and water, under standard temperature conditions ($22 \pm 2\,^{\circ}C$) and a light-dark (12:12) cycle. All animal care and experimental procedures were performed according to the requirements of the National Institutes of Health guide for the care and use of Laboratory animals, the Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation, and were approved by Bioethics Committee of Xuanwu Hospital of Capital Medical University.

2.3. Animal grouping and drug administration

The two strains of male mice (SAMP8 and SAMR1) of three different ages were used and assigned to three tranches of the experiments. (1) Early-term treatment tranche (n = 15 per group): SAMP8 mice of 4 months old were treated with different doses of CIG (50, 100, 200 mg/kg/d), Oxiracetam (360 mg/kg/d, as positive control drug), or saline (as model group) until 6 months old; SAMR1 mice of same age received saline (as normal control), or CIG (100 mg/kg/d). (2) Mid-term treatment tranche (n = 12 per group): SAMP8 mice of 8 months old were treated with CIG (100, 200 mg/kg/d), Oxiracetam, or saline until 10 months old; SAMR1 mice of same age received saline, or CIG (100 mg/kg/d). (3) Late-term treatment tranche: SAMP8 mice of 12 months old were treated with CIG (200 mg/kg/d; n = 15), or saline (n = 18); SAMR1 mice of same age (n = 12) received saline. These three different experiments were conducted successively, and better dose of CIG were chosen in the next experiment to reduce animal numbers (Fig. 1).

CIG and Oxiracetam were dissolved in normal saline and administered intragastrically once a day. Oxiracetam has been applied to patients suffering AD clinically as a nootropic agent [22,23]. In this study it was used as a positive control drug [24]. SAMR1 control group and SAMP8 model group received an equal volume of normal saline. The behavior tests were conducted two months after treatment. The treatment were continued during the behavior tests till the mice were sacrificed.

2.4. Morris water maze task

The procedure of Morris water maze (MWM) test was described previously [25]. The maze consisted of a dark grey tank 120 cm in diameter, filled with 24–26 °C water. The escape platform consisted of clear plexiglas, had a diameter of 9.5 cm and remained in the center of northwest quadrant throughout the training period. During the first day of training, mice were acclimated to the task with the platform placed in the pool. The hidden platform version of the MWM was performed on day 2 to day 6. The whole task lasted for 6 days, with 2 trails conducted at the same time per day. The day after the last training session, mice were subjected to a probe trial session in which the platform was removed from the pool. The mice were allowed to swim for 60 s to search for it. The performance was recorded by a video camera, and analyzed by a computerized video system.

2.5. Object recognition test

The procedure for the object recognition test (ORT) was previously described [26]. The observation arena, with the size of 45 cm × 35 cm × 20 cm, was made of white cast plastic, located in a testing room dimly lit by a constant illumination of about 40 lx in the test arena. The objects chosen are triple copies of cuboid blue plastic blocks (A; 4.5 cm × 4.5 cm × 5 cm), and white cylinderical plastic bottle (B; height: 7 cm; diameter: 4 cm) filled with water to prevent mice from moving. According to our screening test, these two kinds of objects elicited roughly the same exploration time relative to each other. The test lasted for 3 days. On day 1, mice were placed in the empty arena for 10 min to habituate the animals to the stimuli. On day 2, two identical objects were placed at opposite sides in each arena and leaving an 8 cm space from the walls, with 29 cm distance between each other. The mice were placed in the middle of each object to start a 10 min object-learning-training. On day 3, one familiar (previously observed) object and one new object were placed in the arena, and the mice were placed in the middle of each object to start a 10 min object recognition test. Object exploration was defined as the orientation where animals snout toward the object and stands out within 2 cm or less from the object. Objects were cleared with ethanol after each individual trail to equate olfactory cues. The time spent exploring the familiar object and the new object during days 2 and 3 was observed visually and recorded. The time measured was used to calculate a memory discrimination index (DI): $DI = (N - F)/(N + F)$, where N is the time spent exploring the new object and F is the time spent exploring the familiar object [27]. Higher DI is considered to reflect better memory ability.

2.6. Rotating rod test

Rotating rod test (RRT) was used to evaluate the motor performance of the mice and carried out as previously described [28]. A rota-rod apparatus consisted of six rotating drums (3 cm diameter) was used. All animals underwent a 3-day training program on a rotating rod in a speed of 10 rotations per minute (rpm). During the period, the animals were trained three times for 5 min in each trial. On the experimental day, animals were placed individually on the rotating rod (30 rpm) with a fixed cut off time (180 s). The time spent walking on the rod without falling was recorded as the latency to fall off. If a mouse remained on the rod for more than 180 s, its endurance time was recorded as 180 s. The test was performed five times, and the average of the results was calculated.