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Beneficial effects of asiaticoside on cognitive deficits in senescence-accelerated mice

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ABSTRACT

The effect of asiaticoside isolated from *Hydrocotyle sibthorpioides* (AHS) on the promotion of cognition in senescence-accelerated mice (SAMP) was evaluated. Six-month old male SAMP8 mice were orally administered 20, 40 or 80 mg/kg AHS daily for three months. SAMR1 mice were used as a "normal aging" control. The results showed that treatment with AHS significantly improved learning and memory abilities in behavioral tests. AHS-treated mice showed higher antioxidant enzyme activity and lower lipid oxidation in serum compared with untreated SAMP8 mice. Mechanistically, studies showed that AHS markedly reduced the content and deposition of β-amyloid peptide (Aβ) by inhibiting the expression of mRNA for amyloid protein precursor, β-site amyloid cleaving enzyme-1 and cathepsin B and promoting the expression of mRNA for neprilysin and insulin degrading enzyme. In addition, AHS significantly increased the expression of plasticity-related proteins including postsynaptic density-95, phosphor-N-methyl-D-aspartate receptor 1, phospho-calcium–calmodulin dependent kinase II, phospho-protein kinase A Catalyticβ subunit, protein kinase Cγ subunit, phospho-CREB and brain derived neurotrophic factor. Furthermore, AHS increased the levels of acetylcholine (Ach), but decreased cholinesterase (AchE) activity. These results demonstrated that AHS administration may prevent spatial learning and memory decline by scavenging free radicals, up-regulating the activity of antioxidant enzymes, decreasing the level of Aβ, ameliorating dysfunction in synaptic plasticity, and reversing abnormal changes in Ach level and AchE activity. Thus, AHS should be developed as a new drug to prevent age-related cognitive deficits.

1. Introduction

Alzheimer's disease (AD), one of the most prevalent age-dependent neurodegenerative disorders, is characterized by mild cognitive impairment at its onset followed by irreversible neuronal degeneration and dementia in later stages [1]. Approximately 5% of the population aged 65 and over are estimated to be affected by AD [2]. This projection certainly indicates a problem of considerable magnitude, particularly in terms of the number of patients suffering, the affected relatives and the negative socioeconomic outcomes of AD. Impairment of short-term memory, due to neuronal dysfunction and degeneration in the hippocampus and amygdala, marks the first stage in disease progression. Neuropathological hallmarks of AD include the deposition of β-amyloid (Aβ)-containing senile plaques and the presence of intracellular neurofibrillary tangles in hippocampal and cerebral cortical regions [3,4]. The pathogenic mechanisms underlying AD include impaired cholinergic function, increased oxidative stress, induction of the amyloid cascade (i.e., Aβ deposition and plaque formation), deficiencies in steroid hormones, and the appearance of glutamate-mediated

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excitotoxicity [2]. Of all these mechanism, the amyloid cascade hypothesis, which proposes a pivotal role for Aβ in the pathogenesis of AD, is the most widely accepted by investigators in this field [5]. Although the currently available drugs for dementia, such as acetylcholinesterase inhibitors, provide effective temporary treatment of memory dysfunction, they do not prevent or reverse the underlying neurodegeneration [6]. Research has shown that deterioration of memory begins prior to the onset of old age in animals, including humans [7]. Thus, it is extremely important to identify treatments that can prevent or retard AD-related memory decline and to explore preventive mechanisms to delay the onset of memory deterioration [8].

Herbal medicines have been used to treat AD, and many are now being collected and examined in an attempt to identify possible sources of anti-AD therapeutics [9]. Natural compounds, because of their structural diversity, provide a good opportunity to screen for anti-AD agents. Asiaticoside, a pentacyclic triterpenoid saponin, has been described to have antiulcer, antioxidant and anti-inflammatory activities [10]. Asiaticoside also offers protection against chemical-induced hepatotoxicity [11]. Some studies demonstrated that treatment with asiaticoside might induce antidepressant-like effects [12], and attenuate neurotoxicity induced by 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP) in a rat model of Parkinsonism [13]. Moreover, recent studies indicated that asiaticoside has shown to rescue B103 rat neuroblastoma cells against $A\beta_{25-35}$ and H_2O_2-induced neurotoxicity [14]. Based on these reports, it would be of great interest to determine the effects of asiaticoside on cognitive deficits. In the present study, we investigated the effect of administering asiaticoside isolated from *Hydrocotyle sibthorpioides* (AHS) by oral gavage for 3 months on learning and spatial memory loss in SAMP8 mice. The molecular mechanisms involved in the prevention of learning and spatial memory loss were also studied.

Cholinesterase inhibitors are most widely used to treat AD. Their effect is to ameliorate symptoms without achieving permanent improvement. Huperzine A (9-amino-13-ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3.8)]trideca-3(8),6,11-trien-5-one) is a lycopodium alkaloid isolated from the moss *Huperzia serrate* and acts as a potent, highly specific and reversible inhibitor of acetylcholinesterase, with a better therapeutic index than physostigmine and tacrine [15]. In this study, huperzine A was used as positive control.

2. Materials and methods

2.1. Drugs and chemicals

H. sibthorpioides was purchased from Nanning Qianjinzi Chinese Pharmaceutical Co. Ltd (Nanning, China). Voucher specimen (HSL2011091327) was identified by Q.F. Huang in the First Affiliated Hospital of Guangxi Traditional Chinese Medicine University and deposited in the herbarium of the Department of Pharmacology of Guangxi Medical University.

Huperzine A was purchased from Yuzhong Drug Manufactory (Henan, China). Malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-PX) kits were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

2.2. Preparation of asiaticoside from H. sibthorpioides (AHS)

The powder of dried plant of *H. sibthorpioides* (10 kg) was extracted with 80 l 80% ethanol by filtration. The solvent was evaporated under a vacuum to obtain 893.7 g crude extract, which was extracted with petroleum ether, $CHCl_3$ and water-saturated *n*-butanol successively. The water-saturated *n*-butanol (367.2 g) was dissolved in MeOH and filtered through a syringe filter (0.45 μm). The filtrate yielded a yellow powder after concentration, which was purified by recrystallization in MeOH to yield the crude saponin (195.4 g). The crude saponin was then subjected to chromatography on a silica gel column (200–300 mesh, Yantai, PR China; Ø10 cm × 300 cm) eluting with a gradient mixture of $CHCl_3$ and MeOH (0–100% MeOH, 2500 ml each fraction). The eighth fraction yielded a white crystal after concentration, which was purified by Sephadex LH-20 and preparative HPLC to produce compound (81.6 g). Its structure was elucidated on the basis of physicochemical properties and spectral data: mp 231–233 °C; IR (KBr, ν/cm^{-1}) δ: 3416, 2927, 1734, 1638, 1456, 1379, 1270, 1061, 961; ESI-MS m/z: 981.5 $[M + Na]^+$; ^1H NMR(300 MHz, DMSO-d_6) δ: 0.85, 0.89, 1.05, 1.07, 1.10, 1.17; ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 125.6, 138.1, 175.9, 104.8, 102.4, 95.3. The results showed that the compound is asiaticoside, with its molecular formula and molecular weight being $C_{48}H_{78}O_{19}$ and 959.12, respectively. Its chemical structure was shown in Fig. 1. The compound was normally stored at 4 °C. It was dissolved in distilled water and diluted with physiologic saline for test in animal.

2.3. Animals and treatment

Six-month-old male senescence-accelerated mouse prone/8 (SAMP8) and senescence-accelerated mouse resistant/1

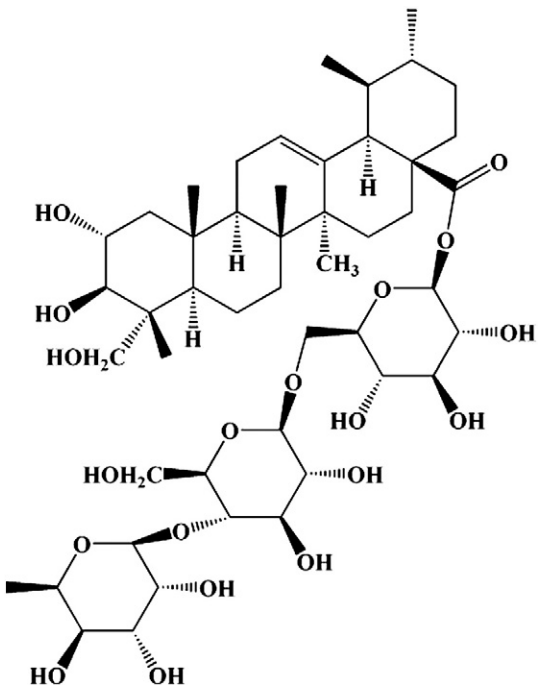

Fig. 1. Chemical structure of asiaticoside.