Steroids 119 (2017) 31–35

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Neuroprotective effect and mechanism of daucosterol palmitate in ameliorating learning and memory impairment in a rat model of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form 8 January 2017
Accepted 10 January 2017
Available online 22 January 2017

Keywords:
Daucosterol palmitate
Alzheimer's disease
Learning and memory
Reactive oxygen species
Hippocampal neurons
Synaptophysin

ABSTRACT

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by progressive memory decline and cognitive impairment. Amyloid beta (Aβ) has been proposed as the causative role for the pathogenesis of AD. Accumulating evidence demonstrates that Aβ neurotoxicity is mediated by glutamate excitotoxicity. Daucosterol palmitate (DSP), a plant steroid with anti-glutamate excitotoxicity effect, was isolated from the anti-aging traditional Chinese medicinal herb *Alpinia oxyphylla* Miq. in our previous study. Based on the anti-glutamate excitotoxicity effect of DSP, in this study we investigated potential benefit and mechanism of DSP in ameliorating learning and memory impairment in AD model rats. Results from this study showed that DSP administration effectively ameliorated Aβ-induced learning and memory impairment in rats, markedly inhibited Aβ-induced hippocampal ROS production, effectively prevented Aβ-induced hippocampal neuronal damage and significantly restored hippocampal synaptophysin expression level. This study suggests that DSP may be a potential candidate for development as a therapeutic agent for AD cognitive decline.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized clinically by the progressive memory decline and cognitive impairment. The histopathological hallmarks of AD are the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles. Amyloid beta (Aβ) protein is the principal component of senile plaques, and has been considered as the causative role for the pathogenesis of AD [1].

A growing body of evidence demonstrates that glutamate-induced excitotoxicity has been implicated to the pathogenesis of neurodegeneration in AD [2]. Moreover, Aβ neurotoxicity is mediated by glutamate-triggered excitotoxicity [3,4].

The fruit of *Alpinia oxyphylla* Miq. is an important anti-aging traditional Chinese medicine. Our previous study [5] demonstrated that the fruits extract of *Alpinia oxyphylla* Miq. exhibited significant neuroprotective effect against glutamate excitotoxicity-induced neuronal apoptosis *in vitro*. In our search for active constituents in the fruits of *Alpinia oxyphylla* Miq. which are responsible for neuroprotective effect against glutamate excitotoxicity, the steroid compound daucosterol palmitate (DSP) was isolated (unpublished data).

Based on the fact that Aβ neurotoxicity is mediated by glutamate excitotoxicity and that DSP has neuroprotective effect against glutamate excitotoxicity, it is reasonable to hypothesize that DSP may have the effect in inhibiting Aβ neurotoxicity. To this end, this study was addressed to investigate potential benefit of DSP in ameliorating learning and memory impairment in Aβ-induced rat model of AD, as well as to clarify the molecular mechanism underlying the neuroprotective effect of DSP in ameliorating Aβ-induced learning and memory impairment in rats.

2. Experimental

2.1. Reagents

Synthetic mouse $A\beta_{(1-42)}$ peptide was purchased from Sigma-Aldrich (St. Louis, MO, USA). 2′,7′-Dichlorodihydrofluorescein diacetate, rabbit anti-synaptophysin antibody and rabbit anti-β-actin antibody were purchased from Cell Signaling Technology (Beverly, MA, USA).

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http://dx.doi.org/10.1016/j.steroids.2017.01.003
0039-128X/

2.2. Preparation of daucosterol palmitate (DSP)

The air-dried fruits of *Alpinia oxyphylla* Miq. were obtained from the local medical herbs store. The plant specimen was authenticated by professor Li JM, Department of Traditional Chinese Medicine, Dalian University. The fruits of *Alpinia oxyphylla* Miq. were cut into small pieces, and then soaked with petroleum ether (PE) for three times each of 12 h at room temperature. The residue was extracted with 95% ethanol for three times each of 1 h in reflux condenser at 55–60 °C. The solution was combined and filtered through filter paper. The solvent was removed in rotary vacuum evaporator to yield a condensed extract. The condensed extract was subjected to column chromatography on silica gel and eluted with PE-acetone (20:1 → 8:1) to yield 9 fractions (Fr. A–I). After examining anti-glutamate excitotoxicity activity in cultured neurons in which active fraction had the protective effect against glutamate excitotoxicity-induced neuronal apoptosis, the active fraction Fr. H was then recrystallized with methanol to yield a active compound. This compound was identified as daucosterol palmitate (DSP) (Fig. 1) by comparison of its EI-MS, ^{1}H NMR and ^{13}C NMR spectral data with that of daucosterol palmitate in the published literature [6]. The purity of isolated DSP is more than 98% as detected by the high-performance liquid chromatography.

2.3. Animals

Male Wister rats (8-week-old, weighing 250–300 g) were obtained from the Experimental Animal Center of Dalian University. The animal breeding was conducted in accordance with the European Communities Council Directive (86/609/EEC) principles for the care and use of laboratory animals. The animal experiment protocols in this study have been approved by the Laboratory Animal Care and Use Committee of Dalian University.

2.4. AD model procedure and drug administration

AD model was established by the bilateral intracerebroventricular injection of $A\beta_{(1-42)}$ as described previously [7]. Briefly, $A\beta_{(1-42)}$ were dissolved in sterile distilled water and incubated for 5 days at 37 °C to aggregate before use. Rats were anesthetized by intraperitoneal injection of chloral hydrate. A total volume of 10 μl of aggregated $A\beta_{(1-42)}$ was injected bilaterally into lateral cerobroventricle using a micro syringe with the help of stereotactic apparatus (at coordinates: AP: 0.8 mm to bregma; lateral: 1.5 mm to sagittal suture and 3.6 mm beneath the surface of the brain). After $A\beta_{(1-42)}$ injection, rats were housed for 7 days for AD symptoms to develop. Sham-operated rats underwent the same operation and injection procedures except that they were injected with the same amount of sterile distilled water.

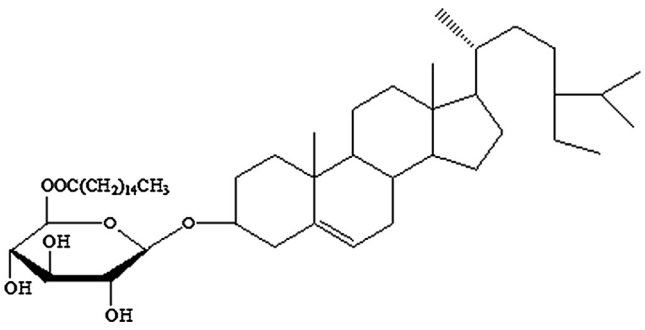

Fig. 1. Chemical structure of daucosterol palmitate (DSP).

Animals were randomly divided into four groups (each of 10 rats): control group, AD model group (AD group), sham group and AD model plus DSP-treated group (AD + DSP group). In AD + DSP group, one day after operation rats were orally administrated with 24 mg/kg/day DSP in distilled water by gavage for consecutive 14 days. Rats of sham group were orally administrated with o.5 ml vehicle (distilled water) by gavage for consecutive 14 days. The dosage of DSP (24 mg/kg/day) and the time of DSP treatment (14 days) used in the present study were chosen based on the results of our pilot experiments in which we used DSP at the dose of 12, 24 and 36 mg/kg/day for consecutive 14 days respectively, and found that DSP shown a dose-dependent effect on ameliorating Aβ-induced learning and memory impairment in rats.

2.5. Spatial learning and memory test

Animal's spatial learning and memory test was performed in a Morris water maze (Model DMS-2, Chinese Academy of Medical Science, Beijing, China). The maze consists of a circular pool which was filled with water. The pool was divided virtually into four equal quadrants, and a transparent platform (escape platform) was hidden 1.5 cm below the surface of the water in the 3rd quadrant. Each rat received three training trials per day for four consecutive days. Rat was placed into one of the four quadrants close to the rim and allowed to swim freely to the platform. Once rat climbed onto the platform, the rat was allowed to remain there for 30 s. If a rat failed to find the platform within 120 s, it was guided to the platform and allowed to remain there for 30 s. If a rat failed to find the hidden platform within 120 s on the fourth day of training, it was dropped from the study. The time required for rat to reach the hidden platform (escape latency) was recorded. The probe test was performed on day 5, the platform was removed and the time rat spent in the target quadrant that previously contained the platform (retention time) was recorded, and the number of times rat crossed over the platform site was also recorded. In addition, the mean swimming speed and path length of rat in pool were recorded. The performance of animals in pool was recorded automatically using a video tracking system. The data was analyzed by the Morris water maze test system (XinTianDi Technology, Beijing, China).

2.6. Preparation of hippocampus sample

After spatial learning and memory test, rats were anesthetized by intraperitoneal injection of chloral hydrate and then sacrificed by decapitation. The hippocampi were dissected immediately from the brain and used for measurement of reactive oxygen species (ROS) production, histological examination and synaptophysin expression, respectively.

2.7. Measurement of ROS production

ROS production was measured based on the oxidation of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) into 2′,7′-dichlorofluorescein (DCF) as described previously [8], with minor modifications. Briefly, hippocampi were homogenized in ice-cold Tris buffer saline in a homogenizer. After the homogenate was diluted with ice-cold Locke's buffer, homogenate was incubated with 5 mM DCF-DA for 60 min at 37 °C. DCF fluorescence was measured in a fluorescence plate reader (Perkin-Elmer LS50B, USA) at the excitation and emission wavelength of 485 and 538 nm, respectively. The ROS level was expressed as the percentage of the DCF fluorescence level in control group whose DCF level was set to 100%.