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Protective effects of linalool against amyloid beta-induced cognitive deficits and damages in mice

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ABSTRACT

Aim: Amyloid-beta (Aβ)-mediated neurotoxicity plays a pivotal role in the pathogenesis of Alzheimer's disease (AD), which induces oxidative stress and apoptosis. Linalool (LI) is a volatile monoterpene showing positive effect in AD treatment. This study was designed to research the protective effect of LI against neurotoxicity and cognitive deficits induced by $A\beta_{1-40}$ in mice.
Main methods: $A\beta_{1-40}$ (4 μg) solution was injected in the bilateral hippocampus to induce cognitive deficits of mice. The protective effects of LI were evaluated by behavioral tests and the related mechanism was further explored by observing the apoptosis and oxidative stress changes in the hippocampus of mice.
Key findings: LI (100 mg/kg, i.p.) administration significantly improved the cognitive performance of model mice in Morris water maze test and step-through test. Meanwhile, LI effectively reversed the $A\beta_{1-40}$ induced hippocampal cell injury in histological examination, apoptosis in TUNEL assay, changes of oxidative stress indicators (SOD, GPX, AChE). Besides, the activated cleaved caspase (caspase-3, caspase-9) was suppressed and Nrf2, HO-1 expression was elevated by LI treatment.
Significance: LI could attenuate cognitive deficits induced by Aβ, and the neuroprotective effect of LI might be mediated by alleviation of apoptosis, oxidative stress depending on activation of Nrf2/HO-1 signaling. We could assume that LI has the potential to be a neuroprotective substance for AD therapy.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia affecting >26.6 million people worldwide, which is clinically characterized by a progressive decline in memory and cognitive function [1]. The key neuropathological hallmarks of the AD brain are extracellular senile plaques induced by accumulation of amyloid β (Aβ) protein and intracellular neurofibrillary tangles [2]. Though the complicated mechanisms underlying AD remain unclear, evidences have indicated that the Aβ induced neurotoxicity and oxidative stress play a key role in its pathogenesis [3–5]. Oxidative stress promotes the production of Aβ, alternatively, the augmentation of Aβ make neurons more susceptible to free radicals, particularly for mitochondrial in neuron [6]. Mitochondrial dysfunction induce energy store exhaustion and ROS overproduction, which contribute to DNA cleavage, protein oxidation and lipid peroxidation, eventually leading to caspase activation and apoptosis [7–9]. Apoptosis is known as programmed cell death, which aggravate the memory and cognitive decline in AD [10]. Thus, it is an important strategy for AD treatment to attenuate Aβ induced oxidative stress and apoptotic neuron death.

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is one of the most important transcription factors having protective response against oxidative stress, [11]. In normal conditions, Nrf2 is sequestered in the cytosol by kelch-like ECH-associated protein 1 (Keap1). Under oxidative stress or regulation, Nrf2 is translocate to bind with antioxidant response element (ARE), activating the expression of defensive genes [12]. Of those genes, heme oxygenase-1 (HO-1) is a vital antioxidant, which exerts beneficial effects in the protection against oxidative injury and regulation of apoptosis in AD [13]. Thus Nrf-2 and HO-1 are considered as important targets for the treatment of AD.

Abbreviations: AD, Alzheimer's disease; Aβ, Amyloid-beta; AChE, acetylcholinesterase; GPX, glutathione peroxidase; HO-1, heme oxygenase-1; LI, linalool; LDH, lactate dehydrogenase; MDA, malondialdehyde; MMP, mitochondrial membrane potential; MWM, Morris water maze; NO, nitric oxide; Nrf2, Nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species; SOD, superoxide dismutase.

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(−)-Linalool (LI) is a major volatile monoterpene component of essential oils from several aromatic plants, such as *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L. and *Coriandrum sativum* L. which were used in traditional medicine [14–16]. LI possesses a variety of bioactivities including anti-inflammatory, antioxidant, anti-tumor, antidepressant, anticonvulsant and antimicrobial [17–21]. Increasing evidences demonstrated that the antioxidant activity of LI is obvious and bring marked benefits for central nervous system (CNS). For example, Linalool exhibited antioxidant properties in H_2O_2 treated guinea pig brain and neuroprotective effect against acrylamide induced neurotoxicity [22, 23]. Besides, linalool also modulates glutamatergic neurotransmission in vitro and in vivo, by interaction with NMDA receptors [24,25]. Thus, we speculated that LI should show neuroprotective effect in AD models.

Recent evidences suggested that LI reverses neuropathological and behavioral impairments in old triple transgenic AD mice and Silexan, which mainly contains LI, has potent neuroprotective effects in scopolamine induced AD mice [26,27]. However, the direct effect of LI on AD model and its mechanism were researched insufficiently. The intrahippocampal $A\beta_{1-42}$ infusion model could induce oxidative damage and neuronal apoptosis in mice to mimic some pathology of AD [28–30]. Therefore, the study was designed to investigate the improvement effect of LI on mice cognitive deficits induced by $A\beta_{1-40}$ and the related mechanism.

2. Materials and methods

2.1. Drug and Aβ preparation

(−)- Linalool (LI, Fig. 1) was obtained from the National institutes for Food and Drug Control (Beijing, China). The enantiomer present in lavender is (*R*)-linalool, which is more woody and lavender-like than (*S*)-form. Amyloid β-protein Fragment 1–40 ($A\beta_{1-40}$) were purchased from Sigma-Aldrich (Lot# SLBL0744V, St. Louis, MO, USA). For intrahippocampal injection, $A\beta_{1-40}$ was dissolved in sterile 0.1 M phosphate-buffered saline (PBS) to get the solution of 1 μg/μL and then incubated at 37 °C for 7 days to obtain the aggregated form of Aβ [31,32].

2.2. Animals

60 male C57BL/6J mice (8 weeks) were provided by the Vital River Laboratories (Qualified No.: SCXK 2012-0001, Beijing, China). All animal were housed in a temperature controlled (25 °C) condition with alternating light/dark cycle (lights, 8:00 AM–8:00 PM), and were given free access to water and diet. All experiments were performed under the approval and supervision of the Academy of Experimental Animal Center of the Institute of Medicinal Plant Development and in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

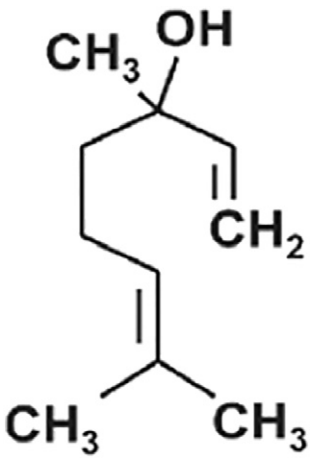

Fig. 1. Molecular structure of linalool.

2.3. Groups and drug administration

The mice were divided randomly into five experimental groups including a control groups, a sham operated group and four $A\beta_{1-40}$-treated group divided into a model group, two LI treated groups (50, 100 mg/kg/d). All mice in $A\beta_{1-40}$-treated group were anesthetized and stereotactically injected aggregated $A\beta_{1-40}$ (4 μL) into the bilateral hippocampus of mice (anterior-posterior position −2.0 mm, medial-lateral position ±1.6 mm, dorsoventral 1.5 mm from bregma). As the previous reports and previous model pretest showed, more plentiful Aβ deposits were observed in model mice through the immunohistochemical staining test, which verified the successful preparation of the $A\beta_{1-40}$ injection AD model [33–35]. The sham group was operated like model preparation but injected with PBS in the hippocampus.

LI was dissolved in a normal saline solution with 2% Tween-80 and 1% DMSO (vehicle) based on the dose of 50 and 100 mg/kg. Drugs were administered intraperitoneally (i.p.) once per day for 7 days before surgery and then for the subsequent 14 days after surgery. The control, sham and model groups were received the same volume of vehicle for 3 weeks.

2.4. Behavioral tests

2.4.1. Locomotor activity test

14 days after surgical operation, the locomotor activity of mice was assessed to preclude the interference of locomotor activity change in the parameters of cognitive function. An open-field computer-aided controlling system was used which consists of four metal tanks (diameter 30 cm, height 40 cm) with a 120 Lux light source and a video camera fixed at the top [36]. 30 min after dosing, each mouse was adapted to the tank for 3 min freely, then the distances travelled in the following 10 min were recorded automatically as the index.

2.4.2. Morris water maze test

The Morris water maze (MWM) test was performed after locomotor activity test to evaluate the spatial learning and memory. The apparatus contains a circular pool filled with water (24–26 °C) and divided into four equal quadrants. A hyaline platform (6 cm diameter, 15 cm height) was submerged 1 cm below the surface in one quadrant (e.g. SE). In navigation experiment which contains four test sessions per day for five days. Each mouse was placed at one quadrant and allowed to find the platform in 60 s. Before and after the swimming, mice were left on platform for 10 s. The escape latency and the escape rate were analyzed by a tracking and image analyzer system. Probing test was conducted with the platform removed the next day after navigation. Mice were released from the quadrant (e.g. NW) opposite from the previous platform location (target quadrant) to receive a 90-s memory retention test. The time in target quadrant and crossing number were recorded and analyzed.

2.4.3. Passive avoidance task

The passive avoidance test was performed as previous method with modification. The apparatus consisted of a white illuminated camber and a dark camber (17 cm × 13.5 cm × 25 cm, respectively) in trough-shape. In training trial, following 180 s habitation each mouse was put into the light chamber to explore with the door opened for 300 s. When it entered the dark chamber, a 0.5 mA electric foot shock (5 s) was delivered. Then mouse was removed from the dark chamber and put back to its home cage. 24 h later, the consolidation trial was performed in the same way as training, and latency to enter the dark chamber and error time were recorded. The latency was recorded up to 300 s.

2.5. Brain sample preparation

After the last behavioral test, all mice were anesthetized and decapitated, and their brains were removed rapidly. 3 whole mice brains in