International Immunopharmacology 23 (2014) 719–725

Contents lists available at ScienceDirect

International Immunopharmacology

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

5-Hydroxymethylfurfural, an antioxidant agent from *Alpinia oxyphylla* Miq. improves cognitive impairment in $A\beta_{1-42}$ mouse model of Alzheimer's disease

Aijing Liu [a], Xu Zhao [a], Huan Li [a], Zhi Liu [a], Bing Liu [a], Xin Mao [a], Lin Guo [a], Kaishun Bi [b], Ying Jia [a,*]

[a] Shenyang Key Laboratory of Active Components of Chinese Medicine Screening and Evaluation, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China
[b] The Engineering Laboratory of National and Local Union of Quality Control for Traditional Chinese Medicine, School of Pharmacy, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 21 July 2014
Received in revised form 11 October 2014
Accepted 28 October 2014
Available online 6 November 2014

Keywords:
Alzheimer's disease
Alpinia oxyphylla Miq
5-Hydroxymethylfurfural
Intracerebroventricular
Slice
Antioxidative

ABSTRACT

5-Hydroxymethylfurfural (5-HMF) is a main effective compound of *Alpinia oxyphylla* Miq. ethanol extract, which showed memory improvement activity against Alzheimer's disease in previous study. In order to identify a potential therapeutic agent, the neuroprotective effects of 5-HMF on impairment of cognition and memory function induced by intracerebroventricular (ICV) injection of $A\beta_{1-42}$ were investigated *in vivo*. The mice were treated with 5-HMF at dose of 15 μg/kg and 150 μg/kg (ICV) for five consecutive days after ICV-$A\beta_{1-42}$. The results showed that 5-HMF significantly ameliorated learning and memory impairment evaluated by the locomotor activity, Y-maze test, and Morris water maze test. Furthermore, 5-HMF significantly inhibited the β-secretase activity, decreased the content of $A\beta_{1-42}$ and malondialdehyde (MDA), and increased the antioxidative enzyme activities including superoxide dismutase (SOD) and glutathione peroxidase (GPx). Results of hippocampus slices showed that neuronal were integrated and regularly arranged in the groups which were administered along with 5-HMF, indicating that 5-HMF could mitigate the degree of neuronal damage. The present study indicated that 5-HMF may serve as a potential therapeutic agent for the treatment of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive, chronic neurodegenerative disease considered as the 5th leading cause of death for those older than the age of 65 and 7th leading cause of death in the United States of America [1,2]. Cognitive decline, intracellular neurofibrillary tangles (NFTs) comprised of phosphorylated tau, extracelluar senile plaque comprised of amyloid-beta (Aβ), inflammation and synaptic dysfunction in the brain are pathological hallmarks of AD [3–5]. Recent studies have demonstrated that the etiology of AD is still not clearly known and current treatments for AD only offer limited symptomatic alleviation; hence, more effective therapeutic agents are urgent [6,7].

Alpinia oxyphylla Miq. has been used as a tonic for kidney and brain in traditional Chinese medicine for thousands of years. In Hainan province, *A. oxyphylla* Miq. is often applied as condiments. Extensive chemical studies revealed that *A. oxyphylla* Miq. contains sesquiterpenes, diterpenes, flavonoids, and diarylheptanoids. In our previous experiment, we have researched that *A. oxyphylla* Miq. ethanol extract ameliorated memory impairment induced by $A\beta_{1-42}$ through the Morris water maze test and passive avoidance response test [8]. A recent study shows that ethanol extract of *A. oxyphylla* Miq. possesses significant neuroprotective activity on glutamate-induced apoptosis in cortical neurons [9]. And another study showed that ethanol extract of *A. oxyphylla* Miq. had therapeutic efficacy for AD through inhibition of tau protein phosphorylation and glycogen synthase kinase 3β generation. [10].

The composition of extract is very multiplex, and a large part of the components are considered to be invalid. In order to find out the most effective ingredients of the herbs, we usually isolate monomer compounds from effective plants and determine the activity. 5-Hydroxymethylfurfural (5-(hydroxymethyl) furan-2-carbaldehyde, 5-HMF) is a main effective compound of *A. oxyphylla* Miq. ethanol extract which can be regarded as the most important heat-induced contaminants occurring in bread and bakery products. Research on this monomer is meaningful for biochemical synthesis, mechanism research, drugs development and clinical application. Modern pharmacological studies have shown that 5-HMF could exhibit multiple biological activities, such as antioxidant, cytoprotective, anti-myocardial ischemia and improving hemorheology effects [11]. The antioxidant effect of 5-HMF is performed on reversing the level of the MDA and the SOD activity in the carbon tetrachloride induced liver injury (aging model) mice and inhibiting hepatocyte oxidative

* Corresponding author at: School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, China. Tel.: +86 24 2398 6933.
E-mail address: jiayingsyphu@126.com (Y. Jia).

http://dx.doi.org/10.1016/j.intimp.2014.10.028
1567-5769/

damage caused by hydrogen peroxide [12]. Based on its reputation of cytoprotective and its antioxidant activity, we hypothesized that 5-HMF which is isolated from *A. oxyphylla* Miq. could mitigate the memory impairment and neurodegeneration in animal model of AD.

2. Materials and methods

2.1. Material

5-HMF was separated from *A. oxyphylla* Miq.. Air-dried powdered fruits (10.0 kg) of *A. oxyphylla* Miq. were exhaustively extracted with 95% ethanol (3 × 10 L), filtrated, concentrated in a rotary evaporator. The 95% ethanolic extract (CMM, 1100 g) was dissolved in distilled water and extracted with petroleum ether, chloroform, ethyl acetate, and n-butanol successively in a separatory funnel. Each organic layer was evaporated to dryness in a rotary evaporator under reduced pressure to yield "PE Fr." (205 g), "$CHCl_3$ Fr." (450 g), "EtOAc Fr." (131 g), and "n-BuOH Fr." (103 g), respectively, while the aqueous phase was lyophilized, "H_2O Fr." (208 g). The $CHCl_3$ Fr. extract (180 g) was applied to a column of silica gel column chromatography (200–300 mesh) and eluted with a gradient of petroleum ether-acetone (100:1, 50:1, 25:1, 10:1, 5:1, 3:1, 1:1) to obtain seven fractions. Fraction 4 (10:1) was further separated by silica gel column (200–300 mesh) eluted with petroleum ether-ethyl acetate to afford 5-HMF which was verified by comparison of the NMR data (Bruker ARX-300, Bruker AV-600) with those in previous reports and compared with the standard sample in our lab [13].

$A\beta_{1-42}$ (Mouse amyloid β Protein Fragment 1–42) was purchased from Sigma-Aldrich (St Louis, MO, USA). Donepezil (DPZ) was obtained from Wanbang Pharmaceutical Company (Zhejiang, China). Commercial kits used for determination of SOD, GSH-px, MDA, Aβ and β-secretase were purchased from Jiancheng Institute of Biotechnology (Nanjing, China).

2.2. Animals

Male Kunming mice, weighing 35 ± 2 g (10-week-old) were purchased from the Central Animal House of Shenyang Pharmaceutical University (Shenyang, China), housed in plastic cages with free access to food and water, and were maintained on a 12 h-light/dark cycle, at a temperature of 23 ± 1 °C. All the studies were reviewed and approved by the Animal Ethical Committee of Shenyang Pharmaceutical University.

2.3. $A\beta_{1-42}$ infused mouse model

$A\beta_{1-42}$ was dissolved and diluted in sterile saline at concentration of 1 mg/mL, and incubated at 37 °C for 5 days before injection. Mice were anesthetized with an intraperitoneal injection of 400 mg/kg chloral hydrate and then fixed in a stereotaxic apparatus [14]. The $A\beta_{1-42}$ group and drugs administered groups were given an injection of $A\beta_{1-42}$ (3 μL) into the left lateral ventricle (AP, −0.5 mm; ML, −1.1 mm; DV, −3.0 mm relative to the bregma), while the sham group were given an injection of saline with the same dose in the same area. The microsyringes were left in the injection site for 3 min to facilitate diffusion of the drugs [15]. After ventricle injection, mice were implanted a 26-gauge stainless steel guide cannula (1 mm) in the right ventricle (AP, −0.2 mm, ML, +1.0 mm, DV, −3.0 mm), which was fixed to the skull with dental cement [16].

2.4. Experimental protocols

The mice were divided into the following six groups of 10 animals each: Control (Ctrl); Sham = ICV-saline + ICV-saline; $A\beta_{1-42}$ group = ICV-$A\beta_{1-42}$ + ICV-saline; 5-HMF (150) group = ICV-$A\beta_{1-42}$ + ICV-5-HMF 150 μg/kg/day; 5-HMF (15) group = ICV-$A\beta_{1-42}$ + ICV-5-HMF 15 μg/kg/day; and DPZ group = ICV-$A\beta_{1-42}$ + ICV-DPZ 15 μg/kg/day. The behavioral procedure timeline is shown in Fig. 1.

2.5. Learning and memory behavioral study

2.5.1. Locomotor Activity

Locomotor activity of each mouse was measured using a Multi-autonomous Activity Instrument with nine activity cages (50 × 50 × 40 cm) using a video-recorded analytical system (Shanghai Jiliang Software Technology Co.Ltd., Shanghai, China). The total distance of movements was evaluated over a 10 min period. The apparatus was placed in a darkened, light and sound attenuated testing room.

2.5.2. Y-maze task

Y-maze is used as a measure of immediate spatial working memory which is a form of short-term memory. The Y-maze is a three-arm maze with equal angles between all arms (30 cm length × 5 cm width × 12 cm high). Mice were initially placed at the end of one arm, and were allowed to move freely through the maze over an 8 min period. The sequence and number of arm entries were recorded manually for each mouse. An actual alternation was defined as entries into all three arms on consecutive choices (ABC, CAB, or BCA but not BAB) [17]. The alternation score (%) for each mouse was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation: % Alternation = [(Number of alternations) / (Total arm entries −2)] × 100 [18].

2.5.3. Morris water maze test

The Morris water maze was selected as a test of spatial learning and memory. A circular water tank (150 cm diameter, 60 cm height) was

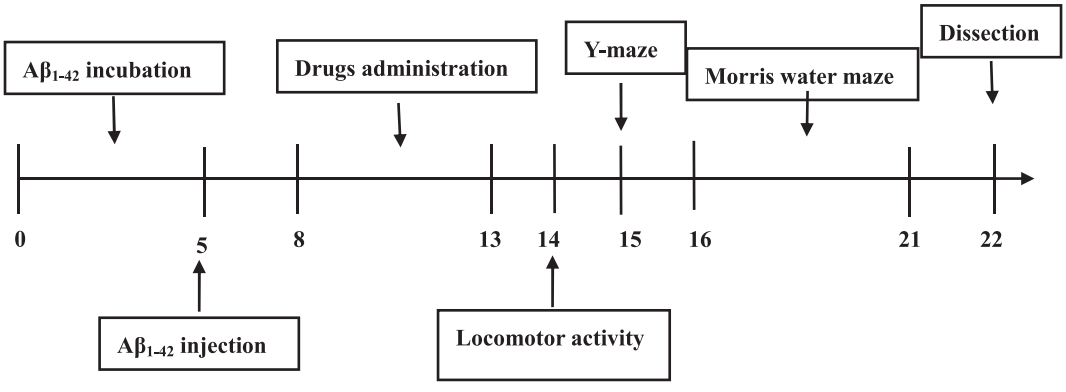

Fig. 1. Behavioral experimental schedule for mice injected with $A\beta_{1-42}$.