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Neuroprotective effects of polygalacic acid on scopolamine-induced memory deficits in mice

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ABSTRACT

Background: *Polygala tenuifolia* Willd is a Traditional Chinese Medicine used for the treatment of learning and memory deficits. Triterpenoid saponins, the main bioactive compounds of *Polygala tenuifolia* Willd, are easily hydrolyzed to polygalacic acid (PA).
Purpose: The present study was undertaken to investigate the neuroprotective effects of PA on scopolamine-induced cognitive dysfunction and to elucidate its underlying mechanisms of action.
Methods: PA (3, 6, and 12 mg/kg) was administered orally to mice for fourteen days, and scopolamine (1 mg/kg) was injected intraperitoneally for fourteen days to induce memory impairment. Memory-related behaviors were evaluated using the Morris water maze. Cholinergic and neuroinflammatory activities were measured in brain tissue. Superoxide dismutase activities, malondialdehyde and reduced glutathione contents were also measured in the brains.
Results: Treatment with scopolamine significantly increased the escape latency time, decreased the number of crossings, and shortened the time spent in the target quadrant, while PA reversed these scopolamine-induced effects. PA significantly improved cholinergic system reactivity, as indicated by decreased acetylcholinesterase (AChE) activity, increased choline acetyltransferase (ChAT) activity, and elevated levels of acetylcholine (ACh) in the hippocampus and frontal cortex. PA also significantly ameliorated neuroinflammation and oxidative stress in mice.
Conclusion: These results suggest that PA might exert a significant neuroprotective effect on cognitive impairment, driven in part by the modulation of cholinergic activity and neuroinflammation.

Introduction

Alzheimer's disease (AD), the most common type of dementia, is characterized by neuronal damage in the hippocampus and other brain regions accompanied by a decline in cognitive function. It has been predicted that the number of people with AD worldwide is estimated to increase from 115 million in 2009 to 135 million by 2050 (Martin et al. 2013). Dysfunction of cholinergic system, including loss of cholinergic cells in the basal forebrain and hippocampus, appears to play a critical role in the pathogenesis of dementia (Becker et al. 1988). The neurotransmitter acetylcholine (ACh), which is synthesized by choline acetyltransferase (ChAT) in cholinergic neurons and hydrolyzed by acetylcholinesterase (AChE) after its release, plays a vital role in central and peripheral control of multiple cognitive processes including learning, memory, and timing (Brandon et al. 2004). The decreased release of ACh following the loss of cholinergic neurons results in learning deficits. Both AChE and butyrylcholinesterase (BChE) hydrolyze ACh rapidly. Thus, overactivity of AChE and BChE can further decrease the ACh level in the brains of patients with AD (Shen 2004). On the other hand, inhibition of AChE and BChE can increase the availability of ACh in the synaptic cleft and is currently the most common treatment strategy for the symptoms of AD (Rijpma et al. 2014).

Various AChE inhibitors, including donepezil, tacrine, rivastigmine, and galamantine, have been used for the treatment of AD for many years; however, the effectiveness of these agents is limited because they lose effectiveness as the disease progresses (Terry and Buccafusco 2003). Therefore, there is a need for more

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; BChE, butyrylcholinesterase; ChAT, choline acetyltransferase; Gala, galantamine; GSH, reduced glutathione; IL-10, interleukin-10; IL-1β, interleukin-1β; MDA, malondialdehyde, MWM, Morris water maze; NC, normal control; PA, polygalacic acid; Scop, scopolamine; SOD, superoxide dismutase.

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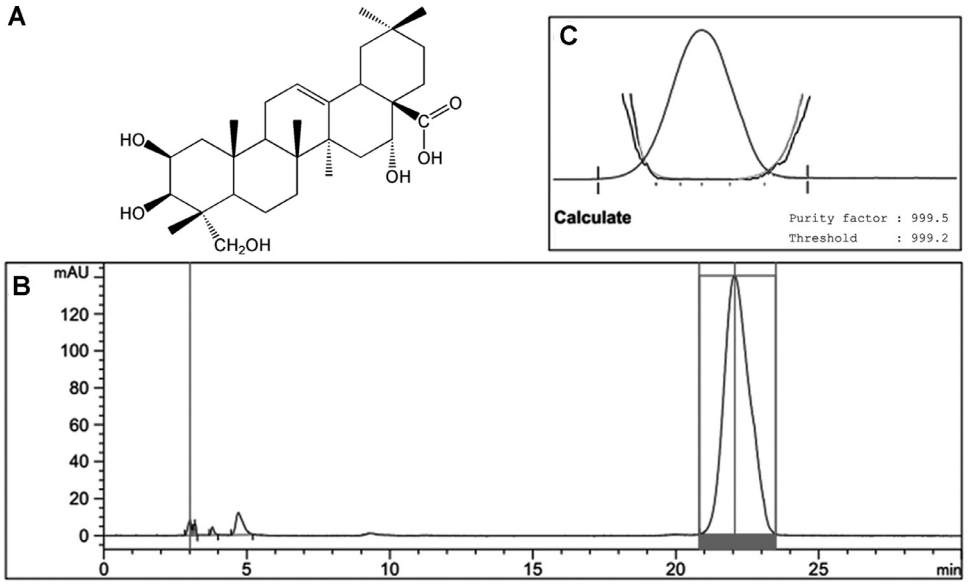

Fig. 1. Chemical structure (A), HPLC chromatogram (B), and purity analysis (C) of PA.

effective cholinesterase inhibitors for AD treatment. Medicinal plants have been used for hundreds of years to treat cognitive decline, especially in Asian countries (Hancianu et al. 2013). For example, the root of *Polygala tenuifolia* Willd (Yuan-Zhi in Chinese) has been widely used in Traditional Chinese Medicine to improve memory function and relieve neurodegenerative illnesses. Triterpenoid saponins, the main bioactive compounds of *Polygala tenuifolia* Willd, have been proven to possess learning and memory enhancing abilities (Lee et al. 2009). However, the triterpenoid saponins are unstable and rapidly hydrolyzed to polygalacic acid (Fig. 1A, PA) (Deng 2009). With this in mind, in this study, we have examined the effect of PA on scopolamine (Scop)-induced memory impairment in mice and investigated the potential mechanisms involved.

Materials and methods

Chemicals and reagents

PA (purity 98%) was supplied by Qingze Pharmaceutical, Inc., Nanjing, China. The peak purity test was performed on Agilent 1260 chromatograph with a diode-array detector (Fig. 1B). Spectra in the range of 210–400 nm were recorded, and the threshold value was calculated based on the absorbance height of each spectrum and a set of noise. In the present work, the PA peak was found pure since the purity factor higher than the threshold (Fig. 1C). Galantamine, the positive control used in these experiments, was obtained from Janssen Pharmaceutical Ltd., Xian, China. Scop was purchased from Sigma-Aldrich Inc., Saint Louis, USA. PA, galantamine, and Scop were dissolved in normal saline solution for the animal experiment. ELISA kits for interleukin-1β (IL-1β) and interleukin-10 (IL-10) were obtained from Abcam Inc, Cambridge, USA. ELISA kit for ChAT was obtained from CUSABIO Inc, Wuhan, China. Kits for malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), AChE, and ACh were purchased from Nanjing Jiancheng Institute, Nanjing, China.

Animals

Adult male Kunming mice (weight 18–22 g), with animal quality certificate number 201501804, were purchased from the Jiangsu University Laboratory Animal Center and maintained at a constant temperature of 23 ± 2 °C and humidity of 50% ± 10% with a 12-h light/dark cycle and free access to normal laboratory food and water. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the ethics committee of the China Pharmaceutical University (CEAE-142, February 12, 2015).

Experimental design

After habituation for seven days, the mice were randomly divided into six groups of ten mice each, as follows: The Scop group was treated with Scop (1 mg/kg) (Park et al. 2012). The galantamine (Gala) group was treated with Scop (1 mg/kg) and galantamine (2 mg/kg) (Gould and Feiro 2005). The PA-3 group was treated with Scop (1 mg/kg) and PA (3 mg/kg). The PA-6 group was treated with Scop (1 mg/kg) and PA (6 mg/kg). The PA-12 group was treated with Scop (1 mg/kg) and PA (12 mg/kg). Dose of PA was determined based on the conversions from clinical adult dose of *Radix Polygalae*. According to the pharmacopoeia of People's Republic of China (State Pharmacopeia Committee of China 2010), the highest dose of *Radix Polygalae* for human adults is 10 g/day. Equivalently, the calculated dose of *Radix Polygalae* based on respective body surface areas for mice is 1.3 g/kg/day. The average content of polygalacic acid in *Radix Polygalae* is 0.86% (Zhao et al. 2010), and so the dose of polygalacic acid for mice is 11.2 mg /kg/day. Therefore, we chose 12 mg/kg/day as high dose, 6 mg/kg/day as middle dose, and 3 mg/kg/day as low dose in this study. The normal control (NC) group was treated with saline at a matched volume. The Scop was injected intraperitoneally to mice for fourteen consecutive days, while galantamine and PA were administrated orally 60 min before Scop injection for fourteen consecutive (Fig. 2).

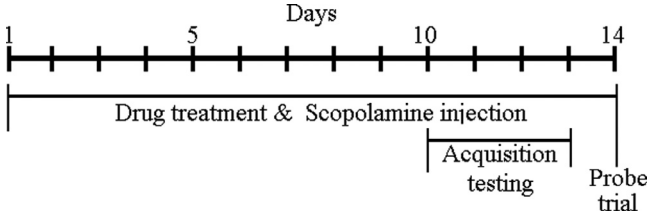

Fig. 2. Animal experimental procedure.