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Research article

Water-soluble ginseng oligosaccharides protect against scopolamine-induced cognitive impairment by functioning as an antineuroinflammatory agent

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ABSTRACT

Background: *Panax ginseng* root is used in traditional oriental medicine for human health. Its main active components such as saponins and polysaccharides have been widely evaluated for treating diseases, but secondary active components such as oligosaccharides have been rarely studied. This study aimed to assess the impact of water-soluble ginseng oligosaccharides (WGOS), which were isolated from the warm-water extract of *Panax ginseng* root, on scopolamine-induced cognitive impairment in mice and its antineuroinflammatory mechanisms.
Methods: We investigated the impact of WGOS on scopolamine-induced cognitive impairment in mice by using Morris water maze and novel object recognition task. We also analyzed the impact of WGOS on scopolamine-induced inflammatory response (e.g., the hyperexpression of proinflammatory cytokines IL-1β and IL-6 and astrocyte activation) by quantitative real-time polymerase chain reaction and glial fibrillary acid protein (GFAP) immunohistochemical staining.
Results: WGOS pretreatment protected against scopolamine-induced learning and memory deficits in the Morris water maze and in the novel object recognition task. Furthermore, WGOS pretreatment downregulated scopolamine-induced hyperexpression of proinflammatory cytokines interleukin (IL)-1β and IL-6 mRNA and astrocyte activation in the hippocampus. These results indicate that WGOS can protect against scopolamine-induced alterations in learning and memory and inflammatory response.
Conclusion: Our data suggest that WGOS may be beneficial as a medicine or functional food supplement to treat disorders with cognitive deficits and increased inflammation.

1. Introduction

Alzheimer's disease (AD) is characterized by the progressive loss of memory and deterioration of cognitive function. The pathological hallmark of AD is the presence of β-amyloid (Aβ) plaques. The accumulation of Aβ may trigger the degeneration of the cholinergic system in the basal forebrain and result in dementia [1]. Acetylcholinesterase inhibitors, which elevate acetylcholine levels in the brain, are accordingly clinically utilized for treating AD [2]; however, this treatment only improves symptoms of AD temporarily without preventing the progression of the disease [3].

Chronic inflammation is an invariant component in the pathogenesis of most neurodegenerative diseases such as AD. After a local injection of Aβ, the inflammatory response occurs in the brain, which is characterized by the upregulation of proinflammatory cytokines and the activation of glial cells [4,5]. Activated glial cells, along with the overexpression of proinflammatory cytokines such as interleukin (IL)-1β) and IL-6 have been associated with the

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lesions of AD as neurotoxic agents [6–8]. Therefore, traditional nonsteroidal anti-inflammatory drugs were considered a potential treatment to prevent or slow the onset of AD in epidemiologic and clinical studies [9,10]; however, significant adverse effects of the drugs at high therapeutic doses were anticipated [11]. Thus, researchers have shifted toward discovering natural compounds that may decrease inflammation in AD [12,13].

Panax ginseng root has been used in traditional oriental medicine for human health, and its neuroprotective effect on different neurologic diseases has been studied [14–16]. The main active components of *Panax ginseng* such as saponins and polysaccharides have been widely evaluated, but secondary active components such as ginseng oligosaccharides have not been widely studied. Water-soluble ginseng oligosaccharides (WGOS), which comprise polymers of 2–14 D-glucose molecules, were recently purified from an aqueous extract of ginseng roots. Studies have been demonstrated that WGOS exert an immunoregulatory effect *in vitro* and *in vivo* [17,18] and display antitumor activity [19]. Thus, we hypothesize WGOS may possess an antineuroinflammatory effect, which may delay the development of neurodegenerative diseases such as AD. To investigate this possibility, we assessed the potential protective effects of WGOS on learning and memory defects and on the hyperexpression of proinflammatory cytokines and astrocyte activation in a scopolamine-induced dementia model.

To date, the only study examining the effect of ginseng oligosaccharides on cognition demonstrated that a mixture of oligosaccharides and peptides from ginseng root can enhance memory in scopolamine-induced dementia in rats; however, the mechanism of this protection was not elucidated [20]. In the current study, we assessed the protective effects of purified ginseng oligosaccharides (i.e., WGOS) against the development of a cognition defect induced by scopolamine. We found that WGOS significantly attenuated scopolamine-induced impairment in the Morris water maze and in the novel object recognition task. In addition, WGOS pretreatment attenuated scopolamine-induced hyperexpression of the inflammatory markers IL-1β and IL-6, and attenuated astrocyte activation in all hippocampal subregions examined. The data from this study shed light on a new potential prophylactic approach toward treating disorders with cognitive deficits and increased inflammation.

2. Materials and methods

2.1. Animals

Male ICR mice (weight, 25–30 g; age, 6–7 weeks old) were obtained from the Animal Center of the College of Basic Medical Sciences in Jilin University (Jilin, China) with approval from the Animal Research Ethics Committee. They were housed 3–4 mice per cage at room temperature (22 ± 2°C) with a 12-h alternating light-dark cycle and free access to food and water. Drug treatment was initiated 5 d after the animals arrived. All behavioral experiments were performed between 8:00 A.M. and 11:00 A.M.

2.2. Drugs and experimental protocol

Water-soluble ginseng oligosaccharides obtained from the water extract of *Panax ginseng* roots [17,18] was provided by Jilin Ginseng Academy at the Changchun University of Chinese Medicine (Changchun, China). Scopolamine hydrobromide was purchased from Shanghai Hefeng Pharmaceutical Co., Ltd. (Shanghai, China). Before the experiments, WGOS was dissolved and scopolamine was diluted with sterile 0.9% saline. All of drugs were administered intraperitoneally (IP).

The mice were assigned to one of six groups, as indicated in Table 1. Three different pretreatments were administered beginning on Day 1 and continued throughout the course of the experiment, as indicated in Fig. 1. The three pretreatments were (1) saline, (2) 40 mg/kg WGOS, or (3) 80 mg/kg WGOS. These dosages for the WGOS were chosen because they produce a robust protection in scopolamine-treated mice, as demonstrated by their performance in the Morris water maze task in our pilot studies. On Day 4 and on all subsequent days, 15 min after the first injection, the mice were administered a second injection with either saline or 3 mg/kg scopolamine. The six groups are the following (presented as pretreatment/treatment): (1) saline/saline (SAL); (2) 40 mg/kg WGOS/saline (WGOS40); (3) 80 mg/kg WGOS/saline (WGOS80); (4) saline/scopolamine (SCO); (5) 40 mg/kg WGOS/scopolamine (WGOS40+SCO); and (6) 80 mg/kg WGOS/scopolamine (WGOS80+SCO). Thirty minutes after the second injection, the mice underwent the behavior tests. One subset of mice in each treatment group was tested with the Morris water maze. One day after the conclusion of this test, these mice were administered the same drug treatments before they were sacrificed for quantitative polymerase chain reaction (qPCR) analysis. Another subset of each treatment group was tested with the novel object recognition task. One day after the conclusion of this test, these mice were sacrificed for immunohistochemistry analysis. The timeline of experimental protocols is shown in Fig. 1.

2.3. Behavioral tests

2.3.1. Morris water maze task

The Morris water maze is a circular pool that is 80 cm in diameter and 40 cm high, and filled to a depth of 19 cm with water containing milk and maintained at 25 ± 2°C. The pool was conceptually divided into four equal quadrants. An escape platform, 11 cm in diameter and 18 cm high, was located at a fixed position in the one quadrant and submerged 1 cm below the water surface.

Table 1
The experimental groups and drug treatments

Group	Pretreatment	Experiment treatment[1)]	
		1st injection	2nd injection
SAL	Saline, IP	Saline, IP	Saline, IP
SCO	Saline, IP	Saline, IP	Scopolamine (3 mg/kg), IP
WGOS40	WGOS (40 mg/kg), IP	WGOS (40 mg/kg), IP	Saline, IP
WGOS40+SCO	WGOS (40 mg/kg), IP	WGOS (40 mg/kg), IP	Scopolamine (3 mg/kg), IP
WGOS80	WGOS (80 mg/kg), IP	WGOS (80 mg/kg), IP	Saline, IP
WGOS80+SCO	WGOS (80 mg/kg), IP	WGOS (80 mg/kg), IP	Scopolamine (3 mg/kg), IP

[1)] Three different pretreatments were administered once daily for 3 d. On Day 4 and all subsequent days, the mice were administered two injections. The first injection was the same drug as the pretreatment drug and the second injection, administered 15 min after the first injection, was saline or scopolamine. Thirty minutes after the second injection, the behavior tests were administered to the mice.
IP, intraperitoneal, SAL, saline; SCO, scopolamine; WGOS, water-soluble ginseng oligosaccharides.