Rehmannia glutinosa Ameliorates Scopolamine-Induced Learning and Memory Impairment in Rats

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Many studies have shown that the steamed root of Rehmannia glutinosa (SRG), which is widely used in the treatment of various neurodegenerative diseases in the context of Korean traditional medicine, is effective for improving cognitive and memory impairments. The purpose of this study was to examine whether SRG extracts improved memory defects caused by administering scopolamine (SCO) into the brains of rats. The effects of SRG on the acetylcholinergic system and proinflammatory cytokines in the hippocampus were also investigated. Male rats were administered daily doses of SRG (50, 100, and 200 mg/kg, i.p.) for 14 days, 1 h before scopolamine injection (2 mg/kg, i.p.). After inducing cognitive impairment via scopolamine administration, we conducted a passive avoidance test (PAT) and the Morris water maze (MWM) test as behavioral assessments. Changes in cholinergic system reactivity were also examined by measuring the immunoreactive neurons of choline acetyltransferase (ChAT) and the reactivity of acetylcholinesterase (AchE) in the hippocampus. Daily administration of SRG improved memory impairment according to the PAT, and reduced the escape latency for finding the platform in the MWM. The administration of SRG consistently significantly alleviated memory-associated decreases in cholinergic immunoreactivity and decreased interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) mRNA expression in the hippocampus. The results demonstrated that SRG had a significant neuroprotective effect against the neuronal impairment and memory dysfunction caused by scopolamine in rats. These results suggest that SRG may be useful for improving cognitive functioning by stimulating cholinergic enzyme activities and alleviating inflammatory responses.

Keywords: Scopolamine, memory, cholinergic neurons, proinflammatory cytokines, Rehmannia glutinosa

Alzheimer’s disease (AD), which is characterized by a progressive decline in cognitive functioning due to the degeneration of the cholinergic nervous system, is one of the most common forms of dementia [1]. Cholinergic deficits are a major neuropathological feature associated with memory loss and have been closely correlated with the severity of cognitive dysfunction associated with AD [12]. Scopolamine (SCO), a blocker of muscarinic acetylcholine (ACh) receptors, induces the dysregulation of cholinergic activity and the impairment of memory functioning, resulting in deficits in the learning, acquisition, and short-term retention of spatial memory tasks [9, 30]. The damage caused by repeated SCO-induced reductions in cholinergic activity has been hypothesized to play a role in reducing hippocampal volume, which has often been associated with AD [34]. AD has also been correlated with the loss of cholinergic neurons and decreases in the levels of acetylcholine (ACh) and choline acetyltransferase (ChAT) [19]. Lesions in these pathways result in decreased ACh release and thus cause learning and memory dysfunction [24]. Until now, inhibition of acetylcholinesterase (AchE) has served as a strategy for the treatment of AD, senile dementia, ataxia, and Parkinson’s disease. The drugs approved for the AD therapy act by counteracting the acetylcholine deficits, providing symptomatic relief, improving cognitive functioning, and enhancing the acetylcholine levels in the brain [4, 13].

Inflammation, as well as cholinergic neuronal degeneration, may play a critical role in the pathogenesis of the degenerative changes and cognitive impairments associated with AD. Proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), are upregulated in the AD brain [26]. These cytokines may play a role in several events in the pathological cascade of AD [8]. Although the mechanisms underlying the anti-amnesic effects of most herbal extracts have not yet been fully elucidated, it has been reported that application of herbal extracts or their pharmacological components has improved...
memory-related behavior [18] and activated central ACh functioning through the inhibition of AchE and the activation of ACh synthesis in patients with Alzheimer’s disease [36].

The steamed root of *Rehmannia glutinosa* (SRG), known as *Sook-Ji-Whang* in Korean, has been used in traditional Oriental medicine to treat various cardiovascular, psychostimulant-related, and inflammatory diseases [17]. An aqueous extract from SRG has been reported to inhibit the inflammation-mediated activation of microglia in rats according to the ischemic brain injury model [16, 31]. Several studies have shown that catalpol, an iridoid of SRG, was effective in ameliorating neurodegenerative changes and improving learning and memory [32, 35], suggesting that the anti-inflammatory effects of SRG may derive from its ability to inhibit the degeneration of cholinergic neurons, thereby alleviating deficits in spatial learning ability in the memory-impairment animal model [17].

The aim of the present study was to evaluate the ability of SRG to improve learning and memory of rats exposed to repeated scopolamine-induced memory deficits as measured by performance on the passive avoidance test (PAT) and the Morris water maze (MWM) test. Moreover, we also examined how these effects were related to the cholinergic system and to anti-inflammatory effects to elucidate the neural mechanisms underlying the memory-enhancing activity of SRG.

**MATERIALS AND METHODS**

**Animals**

Adult male Sprague–Dawley (SD) rats weighing 260–280 g were obtained from Samtaco Animal Co. (Seoul, Korea). The rats were housed in a limited-access rodent facility with up to five rats per polycarbonate cage. The room controls were set to maintain the temperature at 22 ± 2°C and the relative humidity at 55% ± 15%. Cages were lit by artificial light for 12 h each day. Sterilized drinking water and standard chow diet were supplied *ad libitum* to each cage during the experiments. The animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23), revised in 1996, and were approved by the Kyung Hee University Institutional Animal Care and Use Committee. All animal experiments began at least 7 days after the animals arrived.

**Preparation of Rehmannia glutinosa Aqueous Extracts**

*Rehmannia glutinosa* (Gaertn.) was purchased from an Oriental drug store (Health Maximum Co., Jucheon, Korea). The classical processing method used to prepare steamed *Rehmannia glutinosa* is "steaming nine times and drying in the sun nine times" [20]. It was produced by steaming the dried roots for 24 h in the GMP workshop of the factory according to Pharmacopoeia of Korea. A voucher specimen of SRG has been deposited at the herbarium located at the College of Oriental Medicine, Kyung Hee University (Code number KH-SRG01 for SRG). The air-dried and crushed materials were added to distilled water, and extraction was performed by heating for 4 h at 100°C. Then the extract was concentrated with a rotary evaporator (EYELA CCA-1110, Tokyo Rikakikai Co., Tokyo, Japan) and dried with a freeze dryer (EYELA FD-800, Tokyo Rikakikai). The collection rate of the final aqueous extracts was 7.5%.

**Experimental Groups**

To develop learning and memory deficits, male rats were intraperitoneally injected at 2 mg/kg body weight with scopolamine hydrobromide (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), dissolved in physiological saline solution, once a day for 14 days. Normal animals received saline injections instead of scopolamine as a vehicle. Different rats in an experimental group were subjected to either behavioral testing or immunohistochemistry. The rats were randomly divided into six groups of seven individuals as follows: normal group (NOR group, n=7), the saline-induced plus 200 mg/kg SRG-treated group (SRG group, n=7), the scopolamine-induced and saline-treated group (SCO group as a control, n=7), the scopolamine plus 50 mg/kg SRG group (SRG50+SCO group, n=7), the scopolamine plus 100 mg/kg SRG group (SRG100+SCO group, n=7), and the scopolamine plus 200 mg/kg SRG group (SRG200+SCO group, n=7). The rats were intraperitoneally administrated with SRG for 14 days, and SRG was dissolved in 0.9% physiological saline. One hour after SRG administration, all rats except for the NOR group received the scopolamine injection. At the 2nd week after scopolamine injection, rats were subjected to the Morris water maze task. The experimental schedule of all drug administrations and behavioral tests are shown in Fig. 1.

**Passive Avoidance Test**

All animals were subjected to a passive avoidance test. The test was basically performed according to the step-through method described previously [27]. The Gemini Avoidance System (SD Instruments., San Diego, CA, USA) was used for this experiment. Basically, the step-through passive avoidance apparatus consists of a two-compartment acrylic box with a lightened compartment connected to a darkened one by an automatic guillotine door. Electric shocks was delivered to the grid floor of both compartments, made of stainless steel rods (3 mm diameter) spaced 1 cm apart, by an isolated shock generator (Behbood Pardaz Co., Ghaem, Iran). First, rats were subjected to acquisition test trials in the apparatus. In this trial, rats were placed...