Ginsenoside Rg3 Alleviates Lipopolysaccharide-Induced Learning and Memory Impairments by Anti-Inflammatory Activity in Rats

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Abstract

The purpose of this study was to examine whether ginsenoside Rg3 (GRg3) could improve learning and memory impairments and inflammatory reactions induced by injecting lipopolysaccharide (LPS) into the brains of rats. The effects of GRg3 on pro-inflammatory mediators in the hippocampus and the underlying mechanisms of these effects were also investigated. Injection of LPS into the lateral ventricle caused chronic inflammation and produced deficits in learning in a memory-impairment animal model. Daily administration of GRg3 (10, 20, and 50 mg/kg, i.p.) for 21 consecutive days markedly improved the LPS-induced learning and memory disabilities demonstrated on the step-through passive avoidance test and Morris water maze test. GRg3 administration significantly decreased expression of pro-inflammatory mediators such as tumor necrosis factor-α, interleukin-1β, and cyclooxygenase-2 in the hippocampus, as assessed by reverse transcription-polymerase chain reaction analysis and immunohistochemistry. Together, these findings suggest that GRg3 significantly attenuated LPS-induced cognitive impairment by inhibiting the expression of pro-inflammatory mediators in the rat brain. These results suggest that GRg3 may be effective for preventing or slowing the development of neurological disorders, including Alzheimer’s disease, by improving cognitive and memory functions due to its anti-inflammatory activity in the brain.

Key Words: Lipopolysaccharide, Memory, Inflammation, Ginsenoside Rg3, Morris water maze, Cyclooxygenase-2

INTRODUCTION

Neuroinflammation, which includes inflammation of the central nervous system (CNS), has been implicated as a common cause of various neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and ischemic stroke (Zipp and Aktas, 2006; Mrak, 2009). Much evidence suggests that neuroinflammatory and neurodegenerative disorders and sustained increases in various pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the CNS are closely correlated with the neuronal damage and cognitive dysfunction primarily associated with progression of AD pathogenesis (Schwab and McGeer, 2008; Mrak, 2009). For example, in AD, the entorhinal cortex and hippocampus appear to be vulnerable to chronic neuroinflammation. However, these regions exhibit a high degree of gli cell activation in the early disease stage but show a great extent of atrophy with disease progression (Deng et al., 2012). It is considered that synaptic damage may occur during the early phase of chronic neurodegeneration and may lead to cognitive impairment and loss of other neuronal function (Deng et al., 2012). Lipopolysaccharide (LPS), a non-infectious component of the outer membranes of gram-negative bacteria, induces a neuroinflammatory response, impairs memory function, and increases oxidative stress by increasing TNF-α and IL-1β mRNA levels in the hippocampus when administered by intraventricular microinjection or chronic infusion (Kitazawa et al., 2005). LPS is a potent stimulator of microglia to produce proinflammatory cytokines within the brain (Sayyah et al., 2003). Cytokine receptors are distributed throughout the brain with high densities in the hippocampus (Yin et al., 2013). Thus, the hippocampus is thought to be particularly vulnerable to immune-related alterations (Bilbo et al., 2005). It is likely that the interaction of proinflammatory cytio-
kines with neuronal elements during development may alter the brain in a manner that makes it more susceptible to LPS-induced hippocampus-dependent memory impairment or synaptic plasticity in rats (Min et al., 2009; Hwang et al., 2011; Yin et al., 2013). The memory dysfunction caused by LPS-induced inflammation has been hypothesized to play an important role in the pathogenesis of the neurodegenerative changes and cognitive and memory impairments are closely associated with AD (Lukiw and Bazan, 2000; Cunningham et al., 2009). In fact, it is well established that LPS-induced inflammation in the hippocampus produces severe learning and memory deficits in a variety of behavioral tasks (Frank-Cannon et al., 2009). Thus, many studies have suggested that chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) prevents cognitive decline in elderly and other individuals diagnosed with AD dementia (Szekely et al., 2008). However, long-term treatment using NSAIDs can cause gastrointestinal side effects and even occasional liver and kidney toxicity (Graupera et al., 2003). These side effects have encouraged the development of new NSAIDs that are safer for long-term treatment (Kel-Ioff et al., 2000). Recent studies have suggested the use of herbal medicines or natural products for treating Alzheimer’s disease-related disorders exhibiting cognitive memory impairment and neuroinflammation (Ho et al., 2011).

Panax ginseng C.A. Mayer and its constituents are frequently used in Korean traditional herbal medicines to help patients recover from fatigue, enhance resistance capabilities against various neurodegenerative disorders and chronic inflammatory diseases, provide various benefits against memory impairment and to strengthen the immune system (Kim et al., 2011). Many studies have been conducted on the mechanisms of action of Panax ginseng and its processed product, Korean red ginseng (RG), and these substances possess multiple pharmacological and anti-AD activities (Tode et al., 1999; Lee et al., 2010). Ginsenoside, the most effective ingredient in ginseng, is responsible for the pharmacological effects of ginseng (Attele et al., 1999). Ginsenoside prevents memory loss by upregulating the plasticity-related proteins in the hippocampus (Zhao et al., 2011; Lee et al., 2012) and improving learning in mice. Several studies have shown that ginsenoside Rb1 may regulate the inflammatory response by stimulating cyclooxygenase-2 (COX-2) activity against amyloid beta- peptide (Aβ) 1-42-induced memory impairment (Wang et al., 2011) and attenuate the symptoms of scopolamine-induced dementia, as shown by improved cholinergic function and increased cognitive function on behavioral tests (Wang et al., 2010). Ginsenoside Rg3 (GRg3), which is the main component of RG, plays a role in modulating inflammatory processes in the brain (Kang et al., 2007). For example, GRg3 reduces COX-2, inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokine expression, including TNF-α and IL-1, induced by LPS or Aβ 1-42 stimulation in vitro (Bae et al., 2006b; Joo et al., 2008). These studies suggest that the proptopanaxadiol (PD) type of RG ginsenoside may be useful for suppressing inflammation in neurodegenerative diseases. Thus, PD-type GRg3 may be effective for alleviating learning deficits in a memory impaired or neuroinflammatory animal models.

The aim of the present study was to evaluate the anti-inflammatory effects of GRg3 on learning and memory functions in rats exposed to LPS-induced neuroinflammation as measured by performance on the step-through passive avoidance test (PAT) and the Morris water maze (MWM) test. We also examined how these effects were related to the molecular modulation of neuroinflammation in terms of the neural mechanisms underlying the memory-enhancing activity of GRg3.

**MATERIALS AND METHODS**

**Animals**

Adult male Sprague-Dawley (SD) rats weighing 200-220 g (6 weeks-old) were obtained from Samtako 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 National Institutes of Health 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.

**Lesion generation and LPS administration**

To develop learning and memory deficits, male rats were induced with a bilateral intracranial injection (right and left side) of a small dose of LPS, according to procedures described previously by Guo et al. (2010), with a slight modification. The entire operation proceeded with the aid of a stereotaxic apparatus (Stoelting Co., Wood Dale, IL, USA) under anesthesia with sodium pentobarbital (50 mg/kg, i.p.). Fifty micrograms LPS dissolved in 10 μl cerebrospinal fluid (CSF) (Sigma-Aldrich Co., St. Louis, MO, USA) was microinjected into the lateral ventricle in the rat brains in all lesion groups. Sham animals as a vehicle control received microinjection of artificial CSF instead of LPS as a vehicle. Artificial CSF consists of 140 mM NaCl, 3.0 mM KCl, 2.5 mM CaCl2 and 1.2 mM Na2HPO4, and maintained at pH 7.4. The lateral ventricle in the stereotaxic coordinate was designated according to the Paxinos and Watson brain atlas (AP: -0.2, L: ±0.3, DV: -6.2 referenced to the bregma; Paxinos and Watson, 1986). Artificial CSF or LPS solution was injected for 5 min at a flow rate of 2 μl/min using 22-gauge Hamilton syringe and microinjection pump (Pump 22; Harvard Apparatus Inc., Holliston, MA, USA). LPS (Escherichia coli, 055:B5) and ibuprofen were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Administration of GRg3 was started 24 h after the lesion generation.

**Experimental groups**

Different rats in an experimental group were subjected to either behavioral testing or immunochistochemistry. Rats were randomly divided into seven groups of six individuals as follows: CSF-injected sham group being regarded as normal (SAL group, n=6), CSF-injected plus 50 mg/kg GRg3-treated group (GRg3 group, n=6), LPS-injected plus saline-treated group (LPS group as a negative control, n=6), LPS-injected plus 10 mg/kg GRg3-treated group (LPS+GRg3 group, n=6), LPS-injected plus saline-treated group (LPS group as a negative control, n=6), LPS-injected plus 10 mg/kg GRg3-treated group (LPS+GRg3 group, n=6), LPS-injected plus 20 mg/kg GRg3-treated group (LPS+GRg3 group, n=6), and LPS-injected plus 50 mg/kg GRg3-treated group (LPS+GRg3 group, n=6), and LPS-injected plus 40 mg/kg ibuprofen-treated (LPS+IBU group as a positive control, n=6).

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