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Garlic active constituent s-allyl cysteine protects against lipopolysaccharide-induced cognitive deficits in the rat: Possible involved mechanisms

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ABSTRACT

Neuroinflammation is known as a risk factor for cognitive deficit and dementia and its incidence increases with aging. S-allyl cysteine (SAC) is the active and main component of aged garlic extract with anti-inflammatory, neuroprotective, and nootropic potential. In this study, the protective effect of SAC against lipopolysaccharide (LPS)-induced cognitive deficit in the rat was investigated. For induction of learning and memory impairment and neuroinflammation, LPS was intraperitoneally injected at a dose of 167 μg/kg for 7 days and SAC was administered p.o. at doses of 25, 50, or 100 mg/kg/day, 30 min after LPS, for seven days. Treatment of LPS-injected rats with SAC at a dose of 100 mg/kg improved spatial recognition memory in Y maze, discrimination ratio in novel object discrimination task, and retention and recall in passive avoidance test. In addition, SAC at the latter dose mitigated lipid peroxidation marker malondialdehyde (MDA) and augmented key antioxidant defensive elements including superoxide dismutase (SOD), catalase and glutathione (GSH) in hippocampal homogenate and lowered acetylcholinesterase activity. Meanwhile, SAC down-regulated hippocampal nuclear factor- < kappa > B, toll-like receptor 4 (TLR4), glial fibrillary acidic protein (GFAP), and interleukin 1β (IL-1β) and up-regulated nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in addition to lowering iba1-immunoreactive intensity in the hippocampus of LPS-injected group. Taken together, SAC administration could mitigate LPS-induced cognitive deficits via attenuation of oxidative stress, neuroinflammation, astrogliosis, and acetylcholinesterase activity.

1. Introduction

Systemic inflammation and ensuing neuroinflammation is known as a risk factor for cognitive deficit and dementia (Bettcher and Kramer, 2014). The elderly are especially susceptible to the adverse effects of infections on cognitive abilities and neuroinflammation incidence increases with aging (Metti and Cauley, 2012). Microglial activation and increased generation of pro-inflammatory cytokines strongly contribute to neuroinflammation (Brites and Fernandes, 2015; von Bernhardi et al., 2015). Inflammation is also observed in the brains of patients affected with Alzheimer's disease and ischemic stroke and a strong association between chronic inflammation and neurodegeneration has been revealed (DeLegge and Smoke, 2008). Lipopolysaccharide (LPS) is an endotoxin isolated from Gram-negative bacteria that stimulates pro-inflammatory signaling cascades through cell membrane proteins like toll-like receptor 4 (TLR4), leading to overproduction of pro-inflammatory cytokines (Sun et al., 2015b). Systemic injection of LPS induces neuroinflammation and amyloidogenesis in the hippocampus (Lee et al., 2013a). LPS-induced neuroinflammation is also associated with cognitive decline (Hsing et al., 2015). Enhanced oxidative stress (Jangra et al., 2016), increased acetylcholinesterase activity (Ming et al., 2015), activation of microglia and astrocytes and enhanced release of proinflammatory mediators like tumor necrosis factor α and interleukin 1β (IL-1β) (Wang et al., 2014; Zhao et al., 2011), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) down-regulation and nuclear factor- < kappa > B up-regulation (Zhou et al., 2015) also occur following LPS exposure.

S-allyl cysteine (SAC) is the active and main component of aged garlic extract (Saravanan and Ponmurugan, 2010). This constituent has been proposed as a promising organosulfur compound exhibiting a multitude of positive actions in cell models and living organisms (Colin-Gonzalez et al., 2015). SAC has been able to ameliorate oxidative stress and potentiate antioxidant defense system (Ashafaq et al., 2012; Javed et al., 2011). SAC has been able to exhibit neuroprotective effect

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in ischemic neuronal injury via Nrf2-dependent antioxidant response element (Shi et al., 2015), retinal protection against kainate neurotoxicity (Chao et al., 2014), preservation of dopaminergic neurons against the toxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium (Garcia et al., 2014) and to ameliorate β-amyloid-induced brain injury (Tsai et al., 2011). Of interest, SAC has displayed anti-apoptotic and anti-inflammatory effect (Colin-Gonzalez et al., 2015). Furthermore, SAC could attenuate oxidative stress-induced cognitive decline in sporadic Alzheimer's disease model (Javed et al., 2011). It has been reported that anti-inflammatory effect of SAC is partly mediated through NF- < kappa > B signaling (Mong and Yin, 2012). Garlic extract components like SAC are able to suppress LPS-induced TLR4 signaling cascade (Youn et al., 2008). These evidences indicate that SAC may be one of the suitable candidates for inhibition of neuroinflammation and prevention of cognitive deficit following LPS exposure. Therefore, this study was designed to evaluate the efficacy of SAC against LPS-induced cognitive deficit in the rat and to explore some involved mechanisms.

2. Materials and methods

2.1. Experimental design

Male albino Wistar rats (Pasteur's institute, Tehran, Iran, 215–270 g) were housed under standard laboratory conditions of temperature (21–23 °C), humidity (40–60%) and 12:12 h lighting and provided with food and water available *ad libitum*. Procedures involving animals and their care were conducted in conformity with the NIH guidelines for the care and use of laboratory animals. The timeline of experimental procedures has been outlined in Fig. 1.

The rats (n=60) were randomly allocated and similarly grouped into 5 groups: control, LPS, SAC25-treated LPS (LPS+SAC25), SAC50-treated LPS (LPS+SAC50), and SAC100-treated LPS (LPS+SAC100). To induce a systemic inflammatory response, LPS from *Escherichia coli* (SigmaAldrich, St Louis, MO, USA; 0111: B4) was diluted in saline and injected intraperitoneally at a dose of 167 μg/kg for seven days. Systemic LPS administration is a widely-accepted model for neuroinflammation induction in rodents with elevation of brain cytokines and microglial activation (Czerniawski et al., 2015; Henry et al., 2008). This dose of LPS was selected from an earlier study (Czerniawski et al., 2015) and is in a range that could disturb memory processes and not impact exploratory behavior (Bassi et al., 2012; Hennigan et al., 2007). Rats in control group were injected with normal saline only. S-allyl cysteine (SAC) (Abcam, USA) was administered p.o. (dissolved in distilled water) at doses of 25, 50, or 100 mg/kg/day, 30 min after LPS, for seven days. Behavioral tests were performed 3 h after LPS administration on each day. All behavioral experiments were carried out from 11:00 to 16:00 by an experimenter blind to groups and treatments. Behavioral tests including novel object recognition, Y-maze and passive avoidance tasks were conducted at week 1, as depicted in Fig. 1. All animals were killed on day-7 after retention and recall trial of passive avoidance test.

2.2. Y-maze task

Y-maze paradigm is a reliable and non-invasive behavioral test to determine spatial recognition memory in rodents via assessment of spontaneous alternation behavior (Kalalian-Moghaddam et al., 2013). The maze composed of three arms and an equilateral triangular central area. All animals were tested once in a randomized order. Rats were placed at the end of one arm and allowed to move freely through the maze for 8-min period. An arm entry was counted when the hind paws of the rat were completely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets (i.e. A, B, C or B, C, A, etc.). The number of maximum spontaneous alternation was then the total number of arms entered-2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries-2). In addition, total number of arm entries was used as an index of general locomotive activity. The maze was wiped clean with 70% alcohol between sessions to diminish odor cues. Y-maze task was conducted on day-2.

2.3. Novel object discrimination (NOD) task

The used protocol of this test has been described before (Stuart et al., 2013). In this experiment, each rat received two consecutive 5 min object exploration trials separated by a 4 h inter-trial interval (ITI). Rats were exposed to two objects during the first (familiarization) trial, and one of the objects was randomly selected and replaced with a third, novel object in the second (choice) trial. During the two trials exploration of each object, defined as sniffing, licking, chewing, or having moving vibrissae while directing the nose toward and ≤1 cm from the object, was separately recorded. Sitting on an object in the absence of any directed interest was not regarded as exploratory activity. The objects and test areas were wiped with 70% (v/v) ethanol between trials to reduce odor cues. The discrimination (D) ratio was calculated as time spent exploring the novel object compared with the familiar object relative to the total time spent exploring all objects, according to the formula: (t [novel]-t [familiar])/(t [novel]+t [familiar]) *100. NOD task was conducted on day-3.

2.4. Passive avoidance test

This test was conducted according to a previous study with some modifications (Roghani et al., 2006) using the shuttle box apparatus (BPT Co., Tehran). It composed of two compartments, i.e. one illuminated and one dark chamber with grid floor connected by a guillotine door. Electric shock was delivered by an isolated stimulator. On the first and second days, each rat was placed into the apparatus and left for 5 min to explore the chambers and to habituate. During the acquisition trial (third day), rats were placed in the illuminated chamber and after a 5 min habituation period, the guillotine door was opened and the latency to enter the dark chamber was recorded. After the rat entering the dark chamber, the door was closed and an electric foot shock (1 mA, 1 s) was delivered to floor grid. On the acquisition day, the initial latency (IL) of entrance into the dark

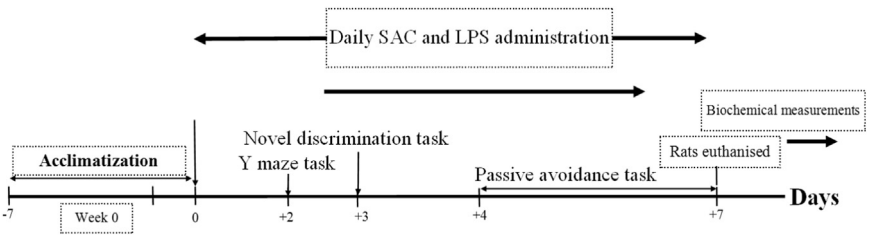

Fig. 1. Schematic experimental protocol for treatments and behavioral tests. Animals received daily treatment of s-allyl cysteine at doses of 25, 50, and 100 mg/kg (i.p.) and lipopolysaccharide at a dose of 167 μg/kg (*i.p.*) for 7 days. Behavioral tests including Y-maze, novel discrimination and passive avoidance tasks were conducted on week 1 post-treatment.