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Research Report

Ligustilide prevents cognitive impairment and attenuates neurotoxicity in D-galactose induced aging mice brain

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ABSTRACT

Ligustilide (LIG) is a principal active ingredient of traditional Chinese medicine, *Radix Angelica sinensis*, which has versatile pharmacological activities including neuroprotection. Previous studies have demonstrated that LIG has beneficial effects on cognition deficits associated with cerebral damage or neurodegenerative disorders. In present study, we investigated the neuroprotective effect of LIG on cognitive impairment and neurotoxicity in the brain of aging mouse induced by D-galactose (D-gal). The aging model mice were induced by subcutaneous (S.C.) injection of D-gal once daily for 8 weeks and LIG (80 mg/kg) was simultaneously administered orally. The Morris water maze (MWM) test was used to assess the spatial learning and memory abilities. The activity of Na^+–K^+-ATPase and the content of lipid peroxidation product malondialdehyde (MDA) in brain were examined. The levels of glial fibrillary acidic protein (GFAP), growth-associated protein GAP-43, and cleaved caspase-3 in brain were also determined by immunohistochemistry. The MWM test showed that LIG administration markedly improved behavioral performance of D-gal treated mice. This action could be partly explained by the results that LIG reduced the level of MDA as well as increased the activity of Na^+–K^+-ATPase in the brain of D-gal induced aging mice. Moreover, LIG significantly raised the expression of GAP-43 and reduced cleaved caspase-3 and GFAP levels in the brain of D-gal treated mice. These results demonstrated that LIG improves D-gal-induced cognitive dysfunction and brain toxicity, which suggests that LIG may be developed as a new medicine for the treatment of aged-related conditions.

Abbreviations: LIG, ligustilide; RAS, *Radix Angelica sinensis*; D-gal, D-galactose; ROS, reactive oxygen species; AGEs, advanced glycation end products; MDA, malondialdehyde; GFAP, glial fibrillary acidic protein; GAP-43, growth associated protein-43; MWM, Morris water maze; PBS, phosphate-buffered saline; H_2O_2, hydrogen peroxide

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1. Introduction

Aging is a complicated multifactorial process that refers to a gradual slow decline in the physiologic function of organisms. Brain aging is an important aspect of the aging process which shows several phenotypes, such as behavioral deficits, a reduction in neurogenesis and lipid peroxidation. Oxidative stress has been proposed to be a major cause in brain aging and age-related neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Castegna et al., 2002; De Iuliis et al., 2005; Lin and Beal, 2006). Excess reactive oxygen species (ROS) can damage cellular macromolecules, such as DNA, proteins, and lipids of cell membranes, thereby disturbing homeostatic within the neurons and astrocytes, and ultimately resulting in cell death (Haripriya et al., 2005; Sohal, 2002). The brain is particularly vulnerable to oxidative damage because of its high oxygen demand, high level of unsaturated lipids, and relative deficiency in anti-oxidative defense mechanisms. Thus, antioxidant therapy is crucial to prevent aging or age-related neurodegenerative disorders. In addition, chronic inflammation was also considered as a major risk factor resulting in aging and age-related diseases (Akbaraly et al., 2013; Chung et al., 2006).

D-galactose (D-gal) is a reducing sugar that can generate ROS in the course of D-gal metabolism when the level of D-gal is greater than that of normal (Ho et al., 2003). Additionally, D-gal can form advanced glycation end products (AGEs) which are not further metabolized and accumulate in cells to aggravate oxidative stress or promote proinflammatory cytokines production (Calcutt et al., 2009; Munch et al., 2012). Chronic administration of D-gal to rodents induces accelerated aging including decline of cognition and motor skills (Cui et al., 2006; Wei et al., 2005). In addition, many recent studies demonstrated that long-term exposure to D-gal induces neurotoxicity in the brain of rodents such as oxidative stress (Zhong et al., 2009), neuronal apoptosis (Tsai and Yin, 2012), decline of neurogenesis (Yoo et al., 2012), astrocytic activation (Lei et al., 2008), and inflammatory response (Lu et al., 2010). D-gal animal model can mimic many characters of nature brain aging or age-related neurodegenerative diseases; therefore, it has been internationally recognized and widely used to study the mechanisms and screen drugs for brain aging (Zhu et al., 2014).

Radix Angelica sinensis (RAS), the root of *Angelica sinensis* (Oliv.) Diels (Umbelliferae), is commonly known as "Danggui" in Chinese, which has been widely used in prescriptions in traditional Chinese medicine since ancient times for replenishing blood, treating abnormal menstruation, and other women's diseases (Mei et al., 1991). Recent studies showed that RAS possesses versatile pharmacological activities including anti-cancer, memory amelioration, radioprotective, and neuroprotective effects (Chen et al., 2013). Ligustilide (3-butylidene-4, 5-dihydrophthalide, LIG), a volatile oil extracted from many umbelliferae plants, has been identified to be the main active ingredient of RAS (Tang et al., 2010). Previous reports have shown that LIG has pleiotropic biological activities such as relaxing smooth muscles (Chan et al., 2007), antiplatelet aggregation (Zhang et al., 2009), and analgesia (Du et al., 2007). The anti-oxidative and anti-apoptotic effects of LIG against hydrogen peroxide-induced injury in PC12 cell (Yu et al., 2008) and anti-inflammatory effects of LIG on lipopolysaccharide-induced inflammation in RAW 264.7 macrophages (Su et al., 2011) or in primary rat microglia (Wang et al., 2010) have been discovered. It has been reported that LIG has significant neuroprotective effects on transient forebrain ischemia in mice (Kuang et al., 2006), permanent focal cerebral ischemia in rats (Peng et al., 2007), and brain ischemia–reperfusion injury in middle cerebral artery occlusion model rats (Peng et al., 2013; Wu et al., 2011). In LIG pharmacokinetic studies, it can be detectable in rat brain after 5–20 min of nasal and oral administration, which indicated that LIG can permeate blood–brain barrier and quickly enter the central nervous system (Guo et al., 2011). Evidences from latest studies demonstrated that LIG can improve cognitive dysfunction associated with chronic cerebral hypoperfusion (Feng et al., 2012) and Alzheimer's disease (Cheng et al., 2011; Kuang et al., 2009, 2014a, 2014b).

Based on the previous findings, we hypothesized that LIG is able to prevent the aging process in D-gal treated mice brain. In the present study, we investigated the protective effect of LIG on cognitive impairment and neurotoxicity in aging mice brain induced by D-gal.

2. Results

2.1. *LIG prevents cognitive impairment in D-gal induced aging mice*

Morris water maze test was used to assess the spatial learning and memory ability of animals. As shown in Fig. 1, all groups of mice improved their performances as indicated by the decreased escape latency and swimming distance across 6 training days. Analyzed by the two-way ANOVA method, significant differences were shown both in mean escape latency and swimming distance between training days ($F=5.570$, $P<0.001$; $F=5.030$, $P<0.001$, respectively) and between treatments ($F=18.579$, $P<0.001$; $F=26.736$, $P<0.001$, respectively) but no interaction between the factors day and treatment ($F=1.066$, $P>0.05$; $F=0.411$, $P>0.05$, respectively). The escape latency and swimming distance in the D-gal group were markedly longer than those in the control group ($P<0.001$) (Fig. 1A and B). It revealed that D-gal-treated mice showed significant cognitive impairment. The D-gal-treated mice that received LIG treatment (D-gal+LIG group) showed a reversal of aforementioned changes in escape latency and swimming distance in the D-gal group ($P<0.001$) (Fig. 1A and B). There was no difference between the D-gal+LIG group and the control group. These results indicated that LIG could improve spatial learning and memory in the D-gal treated mice.

2.2. *Effect of LIG on MDA level and Na^+–K^+-ATPase activity in D-gal treated mice brain*

Our results (Fig. 2A) showed that the level of MDA in mice brain in the D-gal group was significantly higher than that in the control group ($P<0.05$). The increase in MDA level reflecting lipid peroxidation indicates an elevated oxidative