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Research Report

Z-ligustilide activates the Nrf2/HO-1 pathway and protects against cerebral ischemia–reperfusion injury in vivo and in vitro

Bin Peng[a], Peng Zhao[a,b], Ya-Peng Lu[a], Miao-Miao Chen[a], Hua Sun[a], Xiao-Mei Wu[a], Li Zhu[a,*]

[a]Department of Neurochemistry, Institute for Nautical Medicine, Nantong University, 226001 Nantong, China
[b]Obstetrics and Gynecology Hospital, Fudan University, Shanghai 200011, China

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ABSTRACT

Z-ligustilide (LIG), the main lipophilic component of *Radix Angelica sinensis*, has been shown to protect against brain ischemic damage in rodents by oral and intra-peritoneal treatments. The present study aimed to confirm the therapeutic effect of LIG administered intravenously on 2 h middle cerebral artery occlusion (MCAO) and 22 h reperfusion injury in rats since oral administration has low bioavailability, slow absorption and distribution. Moreover, whether LIG activated the NF-E2-related factor 2/ heme oxygenase-1 (Nrf2/HO-1) pathway was also investigated in vivo and in vitro to further elucidate the precise protective mechanisms. In vivo, rats treated intravenously with LIG immediately after the surgery was finished had less neurological dysfunction and smaller infarct volume than that of the vehicle-treated rats. Additionally, LIG promoted Nrf2 nuclear translocation, and further remarkably increased Nrf2 and HO-1 protein expression. In vitro, LIG induced Nrf2 nuclear translocation and up-regulated HO-1 expression in a time-dependent and concentration-dependent manner. Furthermore, LIG treatment reduced cell death induced by OGD, however, the protective action was abolished while Nrf2/HO-1 expression was knockdown by RNA interference. These results noted that intravenous post-treatment with LIG exhibits noticeable neuroprotective properties against brain damage by ischemia–reperfusion and the ability of LIG to activate Nrf2/HO-1 pathway may be partly responsible for it.

1. Introduction

Z-ligustilide (LIG), one of the major active ingredients of *Radix Angelica sinensis*, has been shown to protect against the bilateral common carotid arteries occlusion injury in rodents by oral and intra-peritoneal treatments (Kuang et al., 2006, 2008). Our previous research showed that oral pretreatment with LIG significantly improved behavioral deficit and dose-dependently

Abbreviations: ARE, antioxidant response element; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; LIG, Z-ligustilide; MCAO, middle cerebral artery occlusion; Nrf2, NF-E2-related factor; IKK, I kappa B kinase; OGD, oxygen glucose deprivation; rCBF, regional cerebral blood flow

*Corresponding author. fax: +86 513 85051796.
E-mail address: zhulili65@126.com (L. Zhu).

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reduced the cerebral infarct volume in transient middle cerebral artery occlusion (MCAO) rats (Wu et al., 2011). These findings suggest that LIG has the potential to become a novel agent for treating ischemic stroke. However, the very limited oral bioavailability was reported both in other research (2.6%) and in our study(7.47%), and this may be limited in clinical practice (Supplementary Table 2) (Yan et al., 2007). In addition, the precise mechanisms underlying the neuroprotective effects of LIG are not fully understood.

Reactive oxygen species (ROS) are generated during cerebral ischemia and reperfusion, and are one of the major causes of brain damage (Deb et al., 2010). Some exogenous antioxidants aimed to reduce ROS injury have been studied in the treatment of ischemic stroke. However, they cannot be used widely because of the side effects including DNA damage (Fox et al., 2012). More and more evidence have indicated that stimulation of endogenous antioxidant systems might be an important strategy for achieving neuroprotection on stroke (Jung and Kwak, 2010). NF-E2-related factor (Nrf2), the inducible transcription factor, regulates multiple lines of cellular antioxidant systems that limit oxidative stress during stroke (Alfieri et al., 2011). Under normal conditions, Nrf2 is degraded by Kelch-like ECH-associated protein-1 (Keap1) -dependent pathway. Once activated, Keap1-Nrf2 binding is disturbed and Nrf2 transactivate antioxidant response element (ARE)-driven genes in the nucleus including SOD, GSH-PX and heme oxygenase-1 (HO-1) (Jung and Kwak, 2010). Previous studies have been noted that LIG increases the activity of antioxidant enzyme including SOD in brain tissue, and protects PC12 cells against hydrogen peroxide-induced injury (Kuang et al., 2006; Yu et al., 2007).

In the present study, we first tested the effect of intravenous post-treatment with LIG on brain injury by transient MCAO as it happens in most of human stroke compared to permanent MCAO, and importantly investigated the ability of LIG to activate the Nrf2 pathway in vivo and in vitro to further elucidate the precise protective mechanisms.

2. Results

2.1. *Intravenous post-treatment with LIG protected brain injury by transient MCAO*

To address the potential therapeutic role of LIG in transient MCAO-induced damage, rats were post-treated with a single 5-min intravenous infusion via tail vein. Results showed that infarct volume (%) was 44.04±2.39 in the vehicle-treated group, 37.04±5.33 in the 8 mg/kg group, 25.15±3.07 in the 16 mg/kg group, 17.95±2.67 in the 32 mg/kg group, and 25.74±2.72 in the edaravone group. Statistical analysis revealed that infarct volume in 16 or 32 mg/kg LIG treated group was significantly smaller than that of vehicle-treated rats at 24 h after ischemia (Fig. 1b). But no significant difference in the infarct volume of the 8 mg/kg group was detected compared to vehicle-treated group (Fig. 1b). Besides, both 32 mg/kg LIG and edaravone treatments improved the neurological function at 24 h after ischemia (Fig. 1c).

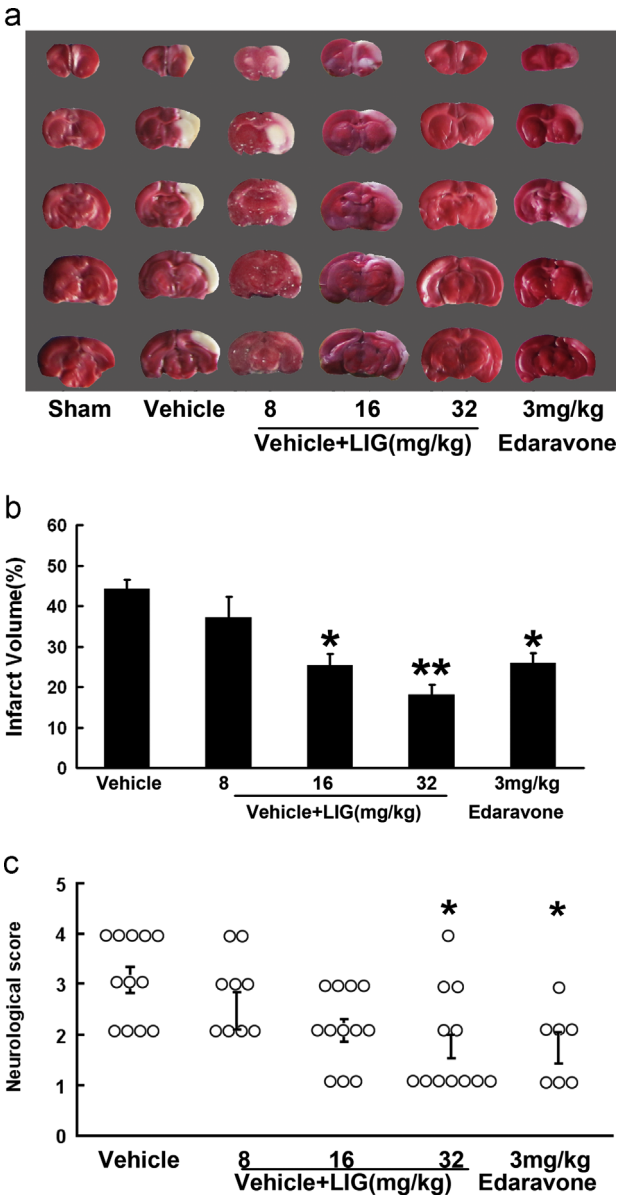

Fig. 1 – Intravenous post-treatment with LIG protected brain injury by transient MCAO. (A) Representative images of TTC-stained sections at 24 h after ischemia. (B) Infarct volumes. Vehicle ($n=12$), 8 mg/kg ($n=9$), 16 mg/kg ($n=12$), 32 mg/kg ($n=12$) and edaravone ($n=7$). Data represent mean±SEM. $^{*}P<0.05$, $^{**}P<0.01$ vs. Vehicle group. (C) Neurological scores were tested at 24 h after ischemia. $^{*}P<0.05$ vs. Vehicle group.

2.2. *LIG attenuated neuronal loss induced by transient MCAO*

With Nissl staining, we found most neurons in the ipsilateral hippocampus, cortex were shrunk and had light color staining (Fig. 2A). Meanwhile, the number of normal neurons in hippocampus CA1 and cortex declined remarkably in vehicle-treated group compared to sham group (Fig. 2B). Interestingly, LIG treatment remarkably attenuated or reversed the neuronal loss.