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Research Report

Ursolic acid promotes the neuroprotection by activating Nrf2 pathway after cerebral ischemia in mice

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ABSTRACT

Background: Oxidative and inflammatory damages have been suggested to play an important role in cerebral ischemic pathogenesis, and provide promising therapeutic strategies for stroke. Nuclear factor-erythroid 2-related factor 2 (Nrf2), a pleiotropic transcription factor, has been shown to play a key role in protecting cells against oxidative injury in cerebral ischemia. In this study, we demonstrated the hypothesis that ursolic acid (UA), a natural pentacyclic triterpenoid acid, isolated from edible plants in the Oleaceae family, a well-known anti-oxidative and anti-inflammatory reagent, protects the brain against ischemic injury by activating the Nrf2 pathway. *Methods*: $Nrf2^{-/-}$ and wild-type (WT) mice were induced into focal cerebral ischemia by transient middle cerebral artery occlusion (MCAO), and received UA treatment immediately after MCAO. The behavioral dysfunction, infarct size, and the expression of Nrf2, HO-1 and inflammatory factors (TLR4 and NF-κB) in ischemic brain were measured at 24 h after stroke. *Results*: UA treatment significantly improved neurological deficit and reduced infarct size in WT mice after MCAO. Administration of UA also decreased the product of lipid peroxidation, promoted the activation of Nrf2 pathway and decreased the expression of TLR4 and NF-KB after stroke in WT mice. However, $Nrf2^{-/-}$ mice demonstrated more severe neurologic deficits, infarct size and inflammatory damage after MCAO, and did not benefit from the protective effect of UA. *Conclusion*: The results indicated that UA protected the brain against ischemic injury in mice by anti-oxidative and anti-inflammatory effects after MCAO. Activation of the Nrf2 pathway contributes to the neuroprotective effects induced by UA in cerebral ischemia.

1. Introduction

Cerebral ischemia, a kind of common and frequently occurring disease, accounts for 70% to 80% of all strokes in the world (Donnan et al., 2008). Worldwide over 15 million people a year, equating to one in every 400 people, suffer a stroke (Kim and Chae, 2009). Ischemic stroke is also a leading cause of mortality equating to 9% of total deaths each year (Rosamond et al., 2008). No medical or surgical therapy to date has been shown to reduce morbidity or mortality after cerebral ischemia (Chiti et al., 2007).

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The current management protocol for cerebral ischemia is limited to supportive medical care. Recanalization following ischemia is the most effective method for treatment of acute cerebral infarct and correction of hypoxia, but paradoxically causes severe cerebral ischemia-reperfusion injury (Amaro and Chamorro, 2011). New paradigms to provide new therapeutic targets for patients outside of thrombolysis window to save the hypoperfused, nonfunctional, but still viable brain tissue surrounding the irreversible infarct core are urgently needed.

Multiple lines of evidence indicate that through targeting a single transcription factor, numerous pathologic cascades can be impeded. For instance, by inhibiting nuclear factor-κB (NF-κB), it is possible to inhibit a broad range of NF-κB-regulated gene products (Wei et al., 2011). Because NF-κB is the master regulator of expression of many proinflammatory genes, its inhibition may ultimately lead to attenuation of many facets of inflammation. Nulear factor-erythroid 2-related factor 2 (Nrf2) is a master regulator that induces a series of cytoprotective factors such as anti-oxidative enzymes, anti-inflammatory and several transcription factors, by activating the antioxidant response element (ARE) pathway, which would be suitable in combating the pathogenic events associated with cerebral ischemia (Ren et al., 2011). Several studies have demonstrated that activation of the redox-sensitive Nrf2 plays a pivotal role in enhancing the endogenous defense mechanism by which the brain protects itself against progressing ischemic damage and recovers from stroke (Wang et al., 2012, 2011). Therefore, therapies targeting the Nrf2 pathway have provided a promising target for stroke research.

Ursolic acid (UA), a natural pentacyclic triterpenoid acid, is one of the major components of certain medicinal plants. Evidence has shown that UA possesses a wide range of biological effects, such as anti-oxidative (Liobikas et al., 2011), antitumor (Wang et al., 2011), and anti-inflammatory (Checker et al., 2012) activities. However, the molecular targets and mechanisms underlying UA are not completely characterized, and the effect of the UA in acute stroke is still unknown. In the present study, we demonstrated the unexplored potential of UA for the prevention and treatment of cerebral ischemic damage, and provided evidence that Nrf2 pathway contributes to the neuroprotective effect of UA in cerebral ischemia. Most importantly, we found that activating the Nrf2 pathway enhanced the anti-inflammatory action of UA in cerebral ischemia.

2. Results

2.1. UA improved neurological deficits in cerebral ischemia in mice

To examine the neuroprotective effect of UA on cerebral ischemia, neurologic deficits were examined and scored at 24 h after MCAO. Compared with mice in the vehicle group, UA-treated mice showed a significant improvement on neurological deficit after treatment (Fig. 1A, $^{*}P<0.05$), suggesting a neuroprotective effect of UA treatment in acute stroke.

2.2. UA reduced infarct volume in cerebral ischemia in mice

The cerebral infarction was detected by triphenyltetrazolium chloride (TTC) staining and is displayed in Fig. 1B. No infarction was observed in the Sham group, while an extensive lesion was developed in both the striatum and the cortex in the vehicle group (Fig. 1B). However, the mice treated with

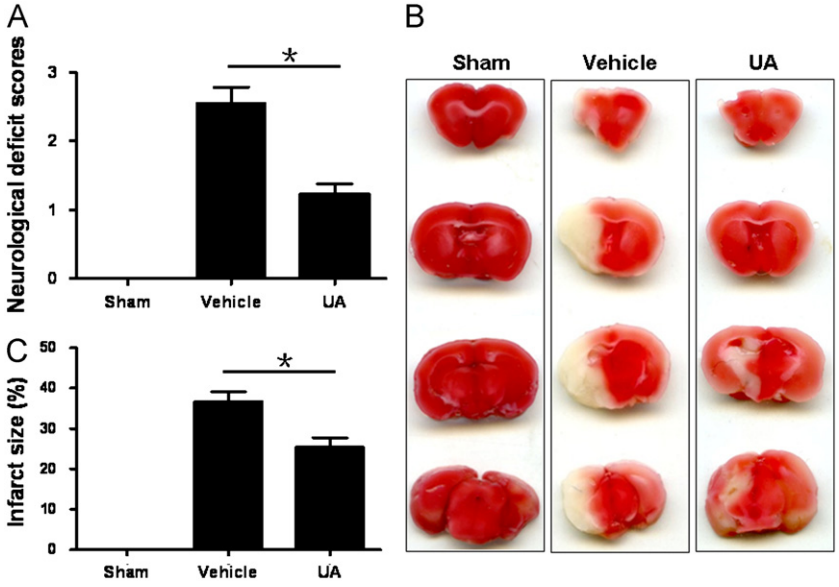

Fig. 1 – UA improved neurological deficit and decreased infarct size in WT mice after MCAO. WT mice were subjected to MCAO and treated with UA 30 min after MCAO. (A) UA decreased neurological deficit scores at 24 h after MCAO ($n=10$). $^{*}P<0.05$. (B) Brain slices stained with TTC 24 h after MCAO. The white section of the slices represented ischemic area. The red section of the slices represented normal tissue. (C) UA decreased infarct size at 24 h after MCAO ($n=6$). $^{*}P<0.05$. vehicle: MCAO+vehicle; UA: MCAO+UA.