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Vitexin reduces hypoxia–ischemia neonatal brain injury by the inhibition of HIF-1alpha in a rat pup model

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ABSTRACT

Previous studies have demonstrated that the early suppression of HIF-1α after hypoxia–ischemia (HI) injury provides neuroprotection. Vitexin (5, 7, 4-trihydroxyflavone-8-glucoside), an HIF-1α inhibitor, is a c-glycosylated flavone that has been identified in medicinal plants. Therefore, we hypothesized that treatment with vitexin would protect against HI brain injury. Newborn rat pups were subjected to unilateral carotid artery ligation followed by 2.5 h of hypoxia (8% O_2 at 37 °C). Vitexin (30, 45 or 60 mg/kg) was administered intraperitoneally at 5 min or 3 h after HI. Vitexin, administered 5 min after HI, was neuroprotective as seen by decreased infarct volume evaluated at 48 h post-HI. This neuroprotection was removed when vitexin was administered 3 h after HI. Neuronal cell death, blood–brain barrier (BBB) integrity, brain edema, HIF-1α and VEGF protein levels were evaluated using a combination of Nissl staining, IgG staining, brain water content, immunohistochemistry and Western blot at 24 and 48 h after HI. The long-term effects of vitexin were evaluated by brain atrophy measurement, Nissl staining and neurobehavioral tests. Vitexin (45 mg/kg) ameliorated brain edema, BBB disruption and neuronal cell death; Upregulation of HIF-1α by dimethyloxalylglycine (DMOG) increased the BBB permeability and brain edema compared to HI alone. Vitexin attenuated the increase in HIF-1α and VEGF. Vitexin also had long-term effects of protecting against the loss of ipsilateral brain and improveing neurobehavioral outcomes. In conclusion, our data indicate early HIF-1α inhibition with vitexin provides both acute and long-term neuroprotection in the developing brain after neonatal HI injury.

1. Introduction

Neonatal hypoxia–ischemia (HI) brain injury remains a leading cause of mortality and severe neurological morbidity in newborns, occurring in approximately 1–3 newborns per 1000 live births (Shankaran et al., 2012). Nearly 50% of infants with a HI insult will die and 25% of the survivors still have to face devastating sequelae, such as cerebral palsy, cognitive and/or sensory deficits, mental retardation, learning disabilities, epilepsy, and other serious neurological diseases (Bass et al., 2004; Ferriero, 2004; Graham et al., 2008; Nelson and Lynch, 2004; Vannucci and Vannucci, 1997). These impairments not only impact quality of life for patients and their families but also place tremendous social and economic burden on healthcare resources (Tian et al., 2013). Consequently, there is an incontestable need to study the mechanisms underlying hypoxic–ischemic brain injury and search for additional possible therapeutic strategies.

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Hypoxia-inducible factor-1 (HIF-1) is a basic helix-loop-helix (HLH) heterodimer, composed of HIF-1α and HIF-1β subunits (Semenza, 1999, 2000a). During hypoxia and ischemia, HIF-1α expression is regulated which then regulates the expression of target genes. HIF-1α is involved in various cellular and systemic adaptive responses to hypoxia or ischemia, with effects on erythropoietin, glucose transporters, glycolytic enzymes, and vascular endothelial growth factor (VEGF) (Bernaudin et al., 2002; Li et al., 2008; Ran et al., 2005; Semenza, 2000b; Wenger et al., 2005).

In previous studies, mice deficient in HIF-1α showed significantly less neuronal cell loss in response to hypoxia than did wild-type mice (Helton et al., 2005). Furthermore, HIF-1α inhibition by preconditioning paradigms with oxygen treatment and another known preconditioning agent 2-methoxyestradiol (2ME2) have been suggested to be neuroprotective following a neonatal hypoxic–ischemic insult (Calvert et al., 2006; Chen et al., 2008). Recent reports have also shown that HIF-1α was inhibited by EPO which subsequently conferred neuroprotection in an in vitro model of hypoxia–ischemia (Souvenir et al., 2014), and pretreatment with berberine promoted PC12 cell survival and inhibited apoptosis under hypoxic conditions by the downregulation of HIF-1α and other pro-apoptotic proteins (Zhang et al., 2012). Thus, searching for new compounds able to inhibit HIF-1α is likely to be beneficial in cerebral hypoxic–ischemic injuries.

Vitexin (5, 7, 4-trihydroxyflavone-8-glucoside) is a c-glycosylated flavone (Fig. 1A) that has been found in medicinal and other plants, such as *Vitex negundo* seeds (Zhou et al., 2009), Pennisetum millet (Gaitan et al., 1989), mung bean (Cao et al., 2011), Passion flower (Zucolotto et al., 2012), mimosa (Zhang et al., 2011), *Phyllostachys nigra* bamboo leaves (Lee et al., 2010), wheat leaves (Moheb et al., 2011), hawthorn (Ma et al., 2010), and chaste tree or chasteberry (Hajdu et al., 2007). Vitexin has recently received increased attention because of its wide range of pharmacological effects including antioxidant (An et al., 2012; Borghi et al., 2013; Kim et al., 2005; Praveena et al., 2013), anticancer (Choi et al., 2006; Papi et al., 2013; Yang et al., 2013; Zhou et al., 2013, 2009), antiviral (Krcatovic et al., 2008; Li et al., 2002), anti-inflammatory (Dong et al., 2011, 2013), anti-nociceptive (Borghi et al., 2013; Demir Ozkay and Can, 2013; Gorzalczany et al., 2011), anti-hypertensive (Je et al., 2014), anti-spasmodic (Gilani et al., 2006; Ragone et al., 2007), and anti-depressant-like actions (Can et al., 2013). In addition, vitexin has neuroprotective effects on both pentylenetetrazole-induced seizures in rats and the neuronal excitotoxicity induced by glutamate (Abbasi et al., 2012; Yang et al., 2014) and ameliorates the memory impairments in rats induced by scopolamine (Abbasi et al., 2013). However, the effect of vitexin on the neonatal brain after a hypoxic–ischemic insult is still not understood. In the present study, we explored the possible neuroprotective role of vitexin, an inhibitor of HIF-1α (Choi et al., 2006), on neonatal hypoxic–ischemic brain injury.

2. Materials and methods

2.1. Experimental animals

Animals were maintained and experiments were conducted in accordance with ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines and was approved by the Institutional Animal Care and Use Committee of Wuhan University (China) (Permit Number: SCXK 2008-0004). All rats (The Animal Biosafety Level 3 Laboratory (ABSL-3), Wuhan University) were grouped randomly and housed under a 12-h light and 12-h dark cycle at 25 ± 2 °C, and in a relative humidity of 60–80%. Animals were fed with food and water available ad libitum throughout the study.

2.2. Animals model of hypoxia–ischemia brain damage

All procedures of the neonatal HI model were adapted from the Rice–Vannucci Model (Rice et al., 1981). Briefly, postnatal Day 7 Sprague-Dawley rat pups of both genders (weighing 13–18 g) were anesthetized by inhalation of diethyl ether. The right common carotid artery was exposed and permanently ligated with 6–0 surgical silk. The wound was then sutured with 4–0 surgical silk. Surgery time for each pup did not exceed 5 min. After recovering with their dams for 2 h, the pups were placed in a 500-mL airtight jar partially submerged in a 37 °C water bath to maintain a constant thermal environment and subjected to 8% O_2 in N_2 for 150 min. Thereafter, animals were returned to their dams. Sham group animals underwent anesthesia and neck incision only.

2.3. Treatment groups and drug administration

172 rat pups of postnatal Day 7 were used in this study and randomly divided into the following groups: Control group (n = 6); Sham group (n = 42); HI group (n = 45); HI + Vitexin group (n = 64); HI + DMOG (n = 15). For drug administration, the HIF-1α inhibitor vitexin was purchased from the Shanghai Tauto Biotechnology (Shanghai, China). Purity: 98% by high performance liquid chromatography (HPLC). To determine the best dosages for effective treatment, vitexin was dissolved in saline and administered intraperitoneally in one of three dosages of 30 mg/kg, 45 mg/kg and 60 mg/kg 5 min after HI. Vitexin (45 mg/kg) was also administered at 3 h after HI to gain insight into the appropriate therapeutic window. Dimethyloxalylglycine (DMOG) (Fig. 1B), an HIF-1α activator was dissolved in saline and administered intraperitoneally (250 mg/kg, Cayman Chemical Company, MI, USA) 5 min after HI (Milkiewicz et al., 2004) as a positive control. The non-treated HI group received the same volume of saline as the treatment group (Fig. 1C).

2.4. Infarct volume measurement

At 48 h post-HI, animals were perfused transcardially with PBS under deep anesthesia. The brains were removed and sectioned into 2 mm slices, then immersed into 2% TTC (2, 3, 5-triphenyltetrazolium chloride monohydrate) solution at 37 °C for 10 min, followed by 4% paraformaldehyde. The infarct volume was traced and analyzed by Image J software (NIH) (Chen et al., 2008).

2.5. Nissl staining

During deep anesthesia, pups were perfused with PBS followed by 4% paraformaldehyde at 48 h or 2 weeks after HI individually. The brains were then removed and Nissl staining followed the standard protocol (Tian et al., 2013).

2.6. Brain water content

At 48 h after HI, animals were deeply anesthetized and the brains were removed and divided into three parts (right hemisphere, left hemisphere, and cerebellum) for water content measurement. Each part was weighed on a high precision balance (Denver Instrument, sensitivity ± 0.001 g) immediately after removal (wet weight) and again after drying in an oven at 105 °C for 24 h as previously described (Chen et al., 2009a). The percentage of water content was calculated as [(wet weight − dry weight)/wet weight] × 100%.