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Full-length Article

Osthole promotes endogenous neural stem cell proliferation and improved neurological function through Notch signaling pathway in mice acute mechanical brain injury

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ABSTRACT

Mechanical brain injury (MBI) is a common neurotrosis disorder of the central nervous system (CNS), which has a higher mortality and disability. In the case of MBI, neurons death leads to loss of nerve function. To date, there was no satisfactory way to restore neural deficits caused by MBI. Endogenous neural stem cells (NSCs) can proliferate, differentiate and migrate to the lesions after brain injury, to replace and repair the damaged neural cells in the subventricular zone (SVZ), hippocampus and the regions of brain injury. In the present study, we first prepared a mouse model of cortical stab wound brain injury. Using the immunohistochemical and hematoxylin-eosin (H&E) staining method, we demonstrated that osthole (Ost), a natural coumarin derivative, was capable of promoting the proliferation of endogenous NSCs and improving neuronal restoration. Then, using the Morris water maze (MWM) test, we revealed that Ost significantly improved the learning and memory function in the MBI mice, increased the number of neurons in the regions of brain injury, hippocampus DG and CA3 regions. Additionally, we found that Ost upregulated the expression of self-renewal genes Notch 1 and Hes 1. However, when Notch activity was blocked by the γ-secretase inhibitor DAPT, the expression of Notch 1 and Hes 1 mRNA was downregulated, augmentation of NICD and Hes 1 protein was ameliorated, the proliferation-inducing effect of Ost was abolished. These results suggested that the effects of Ost were at least in part mediated by activation of Notch signaling pathway. Our findings support that Ost is a potential drug for treating MBI due to its neuronal restoration.

1. Introduction

In recent years, the incidence of mechanical brain injury (MBI) in developing and developed countries is increasing the number of accidents. Not only does primary damage cause destruction of local neurons, MBI secondarily induces a gradual cascade of related events that can result in the death of neurons, including brain edema, inflammatory responses, apoptotic cell death, radical-mediated damage, oxidative stress and dysregulation of calcium homeostasis (Lu et al., 2014; Villapol et al., 2014).

Although there have been improvements in understanding the pathophysiology in MBI, clinical trial drugs aimed at preventing neuronal cells death have failed to show consistent improvement in the patient outcomes (Shlosberg et al., 2010; Geddes et al., 2014; Li and Yang, 2014). The lack of effective therapy to repair injured brain tissue has motivated researchers to focus on stem cells as a potential approach for regeneration. Substantial evidence of neural functional recovery has been provided by exogenous stem cells in MBI models (Ma et al., 2011; Zhang et al., 2013; Blaya et al., 2015). However, there remain many questions to be answered, such as ethical and theoretical issues, the source of stem cells, immune rejection, etc. Recently, interest in promoting regeneration of the MBI has turned to the use of endogenous stem cells (Saha et al., 2012).

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Endogenous neurogenesis in the adult human subventricular zone (SVZ) and subgranular zone (SGZ) of the hippocampus is accepted and increasing evidence indicates that these endogenous neural stem cells (NSCs) may have regenerative and reparative roles in central nervous system (CNS) injury (Sun, 2014). In addition, heightened levels of NSC proliferation have been observed in clinical and animal models of brain injury (Rolfe and Sun, 2015). Furthermore, newly generated cells in the adult brain can differentiate into functioning mature neurons and integrate into neuronal networks (Song et al. (2002)), including those involved in cognitive function (Zhang et al., 2008).

Notch signaling defines a basic pathway that controls cell fate acquisition (Wang et al., 2009). Notch signaling pathways have critical roles in the maintenance, proliferation, and differentiation of NSCs in both developing and adult brains (Imayoshi et al., 2010). Notch is a kind of transmembrane protein receptor, and its large extracellular domain contains 36-tandem epidermal growth factor (EGF)-like repeats and 3 cysteine-rich Notch/Lin-12 repeats (Artavanis-Tsakonas et al., 1999). Notch is activated by binding to ligands of the Delta/Serrate/Lag-2 (DSL) family. Activated Notch undergoes sequential cleavage, which is initially at the S2 site by members of the ADAM family of metalloproteases, and then at the S3 and S4 sites by γ-secretase, resulting in release of the Notch intracellular domain (NICD) and transfer to the nucleus, where it binds to the main effector molecule, the DNA-binding RBP-J transcription factor. Then, it forms a transcription factor to stimulate Hes1/5 transcription and Hes1/5 encodes bHLH nuclear protein, regulating downstream target genes, which in turn regulate neurogenesis (Pratt et al., 2011; Hori et al., 2013; Schwanbeck, 2015). Recent studies have shown that Notch signaling may be conserved in the regulation of adult endogenous NSC proliferation and neurogenesis after brain injury. For example, Notch1 signaling components, including the Notch1 ligands, Jagged1 and Delta1, are co-expressed in the SVZ, SGZ and cerebral cortex of the injured brain with proliferative NSCs (Wang et al., 2009; Tatsumi et al., 2010; Wang et al., 2012). In addition, disruption of Notch1 signaling using a γ-secretase inhibitor interferes with the maintenance and proliferation of NSCs (Chojnacki et al., 2003).

The active compounds in Chinese traditional medicine can regulate the biological characteristics of NSCs and affect neurogenesis. In some searches for new therapeutic drugs for CNS injury, herbs used in traditional medicines for neurogenesis are promising candidates. For example, salvianolic B maintains the self-renewal of NSCs and attenuates the cognitive damage after experimental stroke in rats (Zhuang et al., 2012). Curcumin stimulates the proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus (Kim et al., 2008).

The natural coumarin derivative 7-methoxy-8-isopentenoxycoumarin, also known as osthole (Ost, Fig. 1A), was isolated from medicinal plants, such as *Cnidium monnieri* (L.) Cusson. Ost has anti-inflammatory, -apoptotic, -oxidative stress, and neurotrophic properties that make it promising for therapeutic applications (Ji et al., 2010; Chen et al., 2011; Hu et al., 2013; Gao et al., 2014). Ost exerts neuroprotective effects in experimental models of traumatic brain injury (TBI) via anti-oxidative and -apoptosis activities (He et al., 2012). Additionally, it inhibits immune diseases via modulating inflammatory cytokines (You et al., 2009). Our previous study has indicated that Ost confers neuroprotection against cortical stab wound injury and attenuates secondary brain injury through anti-inflammatory and anti-apoptosis actions (Xia et al., 2015). In addition, Ost attenuates CNS inflammation and demyelination in experimental autoimmune encephalomyelitis (EAE) by promoting the augmentation of brain derived neutrophic factor (BDNF) while suppressing the interferon (IFN)-γ level (Chen et al., 2010). Our previous studies have shown that Ost can promote the proliferation of NSCs in vitro and support hippocampus neurogenesis in APP/PS-1 double transgenic mice (Kong et al., 2015). However, it is unknown whether Ost can promote endogenous NSCs proliferation in mice following MBI.

Based on the known activities of Ost, we hypothesized that Ost might be a potential drug for improving CNS environment, stimulating endogenous NSC proliferation and facilitating structural and functional reconstruction of damaged neural tissues in mice model of MBI. In the present study, the MBI model was established by the needle stabbing method. We assessed the effects of Ost on the proliferation of the NSCs in the SVZ, SGZ and injured cortex and its mechanism was discussed.

2. Materials and methods

2.1. Drug preparation

Ost (No.110822-200305, purity >98%, 244.39 Da; Fig. 1A) was purchased from the National Institute for the Control of Pharmaceutical and Biological products (Beijing, China), dissolved in polyethylene glycol (PEG, less than 10%) and stored at 4 °C (Chao et al., 2010).

5-Bromo-2-deoxyuridine (BrdU; Sigma Chemical Company, St. Louis, MO, USA) was dissolved in water at a concentration of 10 mg/ml and stored at 4 °C. To label proliferative cells, the mice received two daily injections of BrdU (50 mg/kg body weight, ip.) for 3 consecutive days immediately after surgery (Kernie and Parent, 2010; Wang et al., 2007).

γ-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine*t*- butyl ester DAPT (Sigma Chemical Company, St. Louis, MO, UA) could block Notch signal pathway to interfere with the maintenance and proliferation of NSCs (Chojnacki et al., 2003). DAPT was dissolved in DMSO (less than 0.1%) and stored at 4 °C. To block Notch signal pathway, the mice were injected with DAPT (10 mg/kg body weight, ip.) once daily for 7 consecutive days before surgery (Borghese et al., 2010).

2.2. Animals, surgical procedures, and drugs administration

C57BL/6J mice were divided into the following six groups: (1) Sham group (60 mice); (2) Sham + Ost group (60mice) (3) MBI + saline group (60 mice); (4) MBI + Ost group (60 mice); (5) MBI + DAPT group (60 mice); and (6) MBI + Ost + DAPT group (60 mice).

A mouse model of stab wound injury, as previously described (Wang et al., 2007; Lanosa et al., 2011; Takarada-Iemata et al., 2014) with slight modifications, is created to mimic the neuroendoscopy procedure. Briefly, mice were anesthetized with ketamine/xylazine solution (50 mg/kg ketamine and 7.5 mg/kg xylazine in 0.9% NaCl solution), and they were maintained at 37 °C throughout the surgical procedure using a water-heating pad. The mice heads were shaved and disinfected with iodine/alcohol cotton swabs (Casanova et al., 2014); then, the mice were placed in a stereotaxic frame (PTW-1, Chengdu, China). A midline incision was made through the scalp and the skin was retracted. A hole was made over the left cerebral hemisphere using a dental drill until the dura was exposed. A 20-gauge, 1.1-mm diameter needle (BD Nexiva Closed IV Catheter System; BD Biosciences, Franklin Lakes, NJ, USA); with a rigid core was inserted 2.5 mm lateral to the midline, 2.5 mm posterior to the lambdoidal suture, and at a depth of 2.5 mm from the brain surface (Fig. 1B). The needle was left in situ for 20 min before removal. The injury site was covered with sterile bone wax, the skin incision was closed with sutures and mice were allowed to recover in their cages (Villapol et al., 2013). Under the same conditions, the effect of needle insertion on tissue damage was indicated in previous studies, tissue

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