BRAIN RESEARCH 1636 (2016) 130–141

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Nobiletin promotes antioxidant and anti-inflammatory responses and elicits protection against ischemic stroke in vivo

Lan Zhang[a,b,c], Xiangjian Zhang[a,b,c,*], Cong Zhang[a,b,c], Xue Bai[a], Jian Zhang[a,b,c], Xumeng Zhao[a,b,c], Linyu Chen[a,b,c], Lina Wang[a,b,c], Chunhua Zhu[a,b,c], Lili Cui[a,b,c], Rong Chen[b,c], Ting Zhao[a], Yuan Zhao[a,b,c]

[a]Department of Neurology, Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, PR China
[b]Hebei Collaborative Innovation Center for Cardio-cerebrovascular Disease, Shijiazhuang, Hebei 050000, PR China
[c]Hebei Key Laboratory of Vascular Homeostasis, Shijiazhuang, Hebei 050000, PR China

ARTICLE INFO

Article history:
Accepted 4 February 2016
Available online 10 February 2016

Keywords:
Ischemic stroke
Neuroprotection
Nobiletin
Nrf2
HO-1
NF-κB

ABSTRACT

Background: Post-ischemic oxidative stress and inflammation play pivotal roles in the pathogenesis of ischemic stroke and may represent therapeutic targets. Nobiletin (NOB) has been reported to elicit a variety of biological effects through its anti-oxidant and anti-inflammatory properties. Our previous study has demonstrated the beneficial effect of NOB in ischemic stroke, but the underlying mechanisms remain poorly defined. We therefore further investigated the role of NOB in cerebral ischemia and its potential mechanisms.

Methods: Adult male Sprague-Dawley rats were randomly assigned to five groups: Sham (sham-operated+0.05% Tween-80), permanent middle cerebral artery occlusion (pMCAO+0.9% saline), Vehicle (pMCAO+0.05% Tween-80), NOB-L (pMCAO+NOB 10 mg/kg) and NOB-H (pMCAO+NOB 25 mg/kg) groups. Rats were pre-administered intraperitoneally once daily for 3 days prior to ischemia and then received once again immediately after surgery. Neurological deficit, brain edema and infarct volume were evaluated at 24 h after stroke. Immunohistochemistry, western blot and RT-qPCR were used to detect the expression of Nrf2, HO-1, SOD1, NF-κB and MMP-9. SOD1, GSH and MDA were measured by spectrophotometer.

Results: Compared with Vehicle group, neurological deficits and brain edema were relieved in NOB-H group, infarct volume was lessened in both NOB-L and NOB-H groups ($P<0.05$). NOB significantly increased the expression of Nrf2, HO-1, SOD1 and GSH, while decreased the levels of NF-κB, MMP-9 and MDA ($P<0.05$).

Conclusion: NOB may have a neuroprotective effect on cerebral ischemia, and this protection may be through upregulating Nrf2, HO-1 and downregulating NF-κB expression.

*Corresponding author at: Department of Neurology, Second Hospital of Hebei Medical University, 215 Hepingxi Road, Shijiazhuang, Hebei 050000, PR China. Fax: +86 311 66002451.
E-mail address: zhang6xj@aliyun.com (X. Zhang).

http://dx.doi.org/10.1016/j.brainres.2016.02.013
0006-8993/

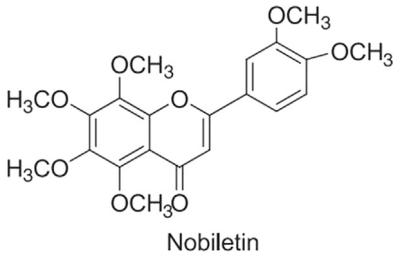
1. Introduction

Ischemic stroke, induced by deprivation of blood flow to regions of the brain resulting in oxygen and glucose supply deficiency, is accompanied with a complex cascade of detrimental events such as glutamate-mediated excitotoxicity, generation of reactive oxygen species (ROS), nitric oxide production, calcium-activated proteolysis (Sahota and Savitz, 2011), eventually leading to neuronal cell death like apoptosis, necrosis, necroptosis and autophagy (Meloni et al., 2011). Evidence demonstrates that the impaired energy metabolism and excessive generation of ROS contribute to neuronal death associated with cerebral ischemia (Zhan and Yang, 2006), which can activate diverse signaling pathways, evoking oxidative stress and inflammatory response. Taking into account the crucial roles of both oxidative and inflammatory mechanisms in the ischemic injury, it may be desirable to explore anti-oxidant and anti-inflammatory agents in combination to combat tissue damage induced by ischemia.

Nuclear factor erythroid 2-related factor 2 (Nrf2), a redox-sensitive transcription factor member of the Cap'n'Collar, regulates numerous anti-oxidant/detoxification genes acting in synergy to remove ROS/RNS through sequential enzymatic reactions (Ishii et al., 2000; Shih et al., 2005). Under basal non-stressed conditions, Nrf2 is sequestered in the cytoplasm by binding to Kelch-like ECH-associated protein 1 (Keap1) to form the Keap1-Nrf2 complex which limits Nrf2-mediated gene expression (Kobayashi and Yamamoto, 2005). Upon exposure of cells to oxidative stress and other potentially damaging stimuli, Nrf2 dissociates from Keap1, translocates into the nucleus to bind antioxidant response element (ARE), and activates ARE-dependent transcription of phase II and antioxidant defense enzymes, such as heme oxygenase-1 (HO-1), superoxide dismutase 1 (SOD1), glutathione S-transferases (GSTs), NAD(P)H quinone oxidoreductase (NQO1) and γ-glutamyl cysteine ligase (γ-GCL) (Kobayashi and Yamamoto, 2005; Satoh et al., 2006). Among phase-II enzymes, HO-1, a ubiquitous and redox-sensitive inducible stress protein, has attracted special attention due to its indirect anti-oxidative effect by degrading heme to biliverdin, free ferrous iron, and carbon monoxide (CO) (Deshane et al., 2005). Moreover, these byproducts of heme degradation have their own significance in essential cellular metabolism and confer generalized endogenous protection against oxidative stress (Berberat et al., 2003; Nakao et al., 2005).

The inflammatory response to brain injury, especially nuclear factor-kappa B (NF-κB) activation, plays a vital role in the pathogenesis of stroke, exerting deleterious effects on the progression of tissue damage (Harari and Liao, 2010). Therefore, inhibition of NF-κB may represent a therapeutic strategy in the acute stage of ischemic stroke. NF-κB is expressed in many cell types in the nervous system (O'Neill and Kaltschmidt, 1997) and is constitutively active in subsets of cells in the cortex and hippocampus of the rodent brain at comparably low levels (Kaltschmidt et al., 1994). Activation of NF-κB signaling is mediated by the upstream kinase inhibitor of kappaB kinase (IKK) which is triggered by hypoxia, ROS, and several inflammatory mediators (Ridder and Schwaninger, 2009). In unstimulated cells, NF-κB resides in the cytoplasm as a complex with inhibitor κB (IκB) proteins that mask their nuclear localization signal. Upon cell activation, IκB is phosphorylated and proteolytically degraded, resulting in NF-κB translocation to the nucleus (Baldwin, 1996). Whenever being activated, NF-κB regulates a wide array of inflammatory mediators, such as interleukin-1β (IL-1β), cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and matrix metalloproteinase-9 (MMP-9), all of which play crucial roles in ischemic damage (Harari and Liao, 2010; Yi et al., 2007; Zheng and Yenari, 2004).

Nobiletin (NOB), a polymethoxylated flavone that commonly presents in citrus peels (Fig. 1), has been recognized to be a promising anti-oxidant and anti-inflammatory agent in the treatment of asthma, colitis and Alzheimer's disease (Cheng et al., 2006; Nakajima et al., 2013; Xiong et al., 2015). With respect to its anti-oxidant properties, pretreatment with NOB protected PC12 cells against hydrogen peroxide(H_2O_2)-induced cytotoxicity by restoring glutathione (GSH) and superoxide dismutase (SOD) contents, diminishing malondialdehyde (MDA) level, and scavenging ROS formation (Lu et al., 2010). A Citrus aurantium extract mainly containing NOB and tangeretin ameliorated ethanol-induced liver injury through enhancing phosphorylation of AMP-activated protein kinase (AMPK) and Nrf2 with several cytoprotective proteins including HO-1, NQO1 and γ-glutamylcysteine synthetase (γ-GCS) in a binge drinking mouse model (Choi et al., 2015). Besides, researchers present evidence that NOB improved the age-related cognitive impairment in senescence-accelerated mouse prone 8 (SAMP8), accompanied by reduction of oxidative stress and tau hyperphosphorylation in the brain of SAMP8 mice (Nakajima et al., 2013). As we know, NOB has profound effects on the immune function and inflammatory cells as determined by numerous studies both in vitro and in vivo. By using a lipopolysaccharide(LPS)-stimulated BV-2 microglia cell model, NOB possessed potent anti-neuroinflammatory capacity, blocked activation of signal pathways underlying microglial activation such as the ERK, JNK, p38 MAPKs pathways, as well as translocation of NF-κB and the subsequent gene expression of iNOS, TNF-α and IL-1β (Cui et al., 2010). Recently another group demonstrated that NOB is the most potent inhibitor of neuroinflammation among eight common tangerine flavonoids (Ho and Kuo, 2014). Additionally, NOB significantly decreased LPS-stimulated expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and IL-8) and MMP-9 expression and pro-MMP-9 secretion in both fetal membranes and myometrium (Morwood and Lappas, 2014). Moreover, for cerebral ischemia, NOB has been proved to suppress delayed neuronal cell death induced by bilateral common carotid arteries occlusion



Nobiletin

Fig. 1 – The chemical structure of nobiletin.