BRAIN RESEARCH 1359 (2010) 216–226

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

DL-3-*n*-Butylphthalide prevents neuronal cell death after focal cerebral ischemia in mice via the JNK pathway

Jimei Li[b], *Yin Li*[b], *Molly Ogle*[b,c], *Xin Zhou*[a,c], *Minke Song*[c], *Shan Ping Yu*[a,c], *Ling Wei*[b,c,*]

[a]*Department of Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC 29425, USA*
[b]*Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29425, USA*
[c]*Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA 30322, USA*

ARTICLE INFO

Article history:
Accepted 19 August 2010
Available online 25 August 2010

Keywords:
DL-3-*n*-Butylphthalide
Ischemic stroke
Apoptosis
Caspase
AIF
Cytochrome *c*
Mitochondria
MAP kinase

ABSTRACT

DL-3-*n*-Butylphthalide (NBP) has shown cytoprotective effects in animal models of stroke and has passed clinical trials as a therapeutic drug for stroke in China. Hence, as a potential clinical treatment for stroke, understanding the mechanism(s) of action of NBP is essential. This investigation aimed to delineate the cellular and molecular mechanism of NBP protection in neuronal cultures and in the ischemic brain. NBP (10 μM) attenuated serum deprivation-induced neuronal apoptosis and the production of reactive oxygen species (ROS) in cortical neuronal cultures. Adult male 129S2/sv mice were subjected to permanent occlusion of the middle cerebral artery (MCA). NBP (100 mg/kg, i.p.) administrated 2 hrs before or 1 hr after ischemia reduced ischemia-induced infarct formation, attenuated caspase-3 and caspase-9 activation in the ischemic brain. TUNEL-positive cells and mitochondrial release of cytochrome *c* and apoptosis-inducing factor (AIF) in the penumbra region were reduced by NBP. The proapoptotic signaling mediated by phospho-JNK and p38 expression was downregulated by NBP treatment in vitro and in vivo. It is suggested that NBP protects against ischemic damage via multiple mechanisms including mitochondria associated caspase-dependent and -independent apoptotic pathways. Previous and current studies and recent clinical trials encourage exploration of NBP as a neuroprotective drug for the treatment of ischemic stroke.

1. Introduction

DL-3-*n*-Butylphthalide (NBP) is a synthesized compound based on the pure component, L-3-*n*-butylphthalide, originally extracted from the seeds of *Apium graveolens* Linn (Chang and Wang, 2003). It is a chiral molecule that has three different stereo isomers known as L-, DL-, and D-NBP. All isomers have shown neuroprotective effects against hypoxia-induced damage (Yan and Feng, 1998). The neuroprotective effects of NBP and possible mechanisms have been explored before (Chong and Feng, 1999a, b; Deng and Feng, 1997; Liu and Feng, 1995; Peng et al., 2008). NBP has protective effects that reduce ischemia-induced brain damage and neuronal cell death, improve cerebral blood flow, decrease brain edema, and preserve the blood–brain barrier (Chong and Feng, 1999a, b; Deng and Feng, 1997; Liu and Feng, 1995; Yan and Feng, 1998; Zhang et al., 2006). NBP also shows beneficial effects in attenuating β-amyloid-induced cell death in neuronal cultures and improving cognitive impairment in an

* *Corresponding author*. Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA 30322, USA. Fax: +1 404 712 1351.
E-mail address: lwei7@emory.edu (L. Wei).

0006-8993/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.08.061

animal model of Alzheimer's disease (Peng et al., 2008; Peng et al., 2010). In addition, NBP has antiapoptotic, antiplatelet, antithrombotic, and anti-inflammatory properties (Chang and Wang, 2003; Peng et al., 2008; Xu and Feng, 2000). However, the molecular mechanism of the effects of NBP remains obscure. For potential clinical applications, the verification of NBP effects in different stroke models is necessary.

Mitochondrial dysfunction is related to necrotic and apoptotic cell death. In apoptosis, mitochondria play a key role in both caspase-dependent and -independent apoptotic processes. The release of cytochrome *c* and apoptosis-inducing factor (AIF) from mitochondria to the cytosol is a clear indication of mitochondrial dysfunction. Cytosolic cytochrome *c* and AIF then lead to caspase activation and AIF nuclear translocation, which are key steps in caspase-dependent and -independent apoptotic cascades, respectively (Kluck et al., 1997; Susin et al., 1999).

Mitogen-activated protein kinases (MAPKs) respond to extracellular stimuli and regulate various cellular activities, such as gene expression, mitosis, differentiation, and apoptosis (Kuida and Boucher, 2004). c-Jun N-terminal kinase (JNK) and p38 MAP kinase are important members of the MAPK superfamily and key modulators of apoptosis (Nishina et al., 2004; Xia et al., 1995). Whether NBP affects the MAPK pathway has not been explored.

In this study, we examined the neuroprotective effects of DL-NBP against apoptosis in cultured neurons and against

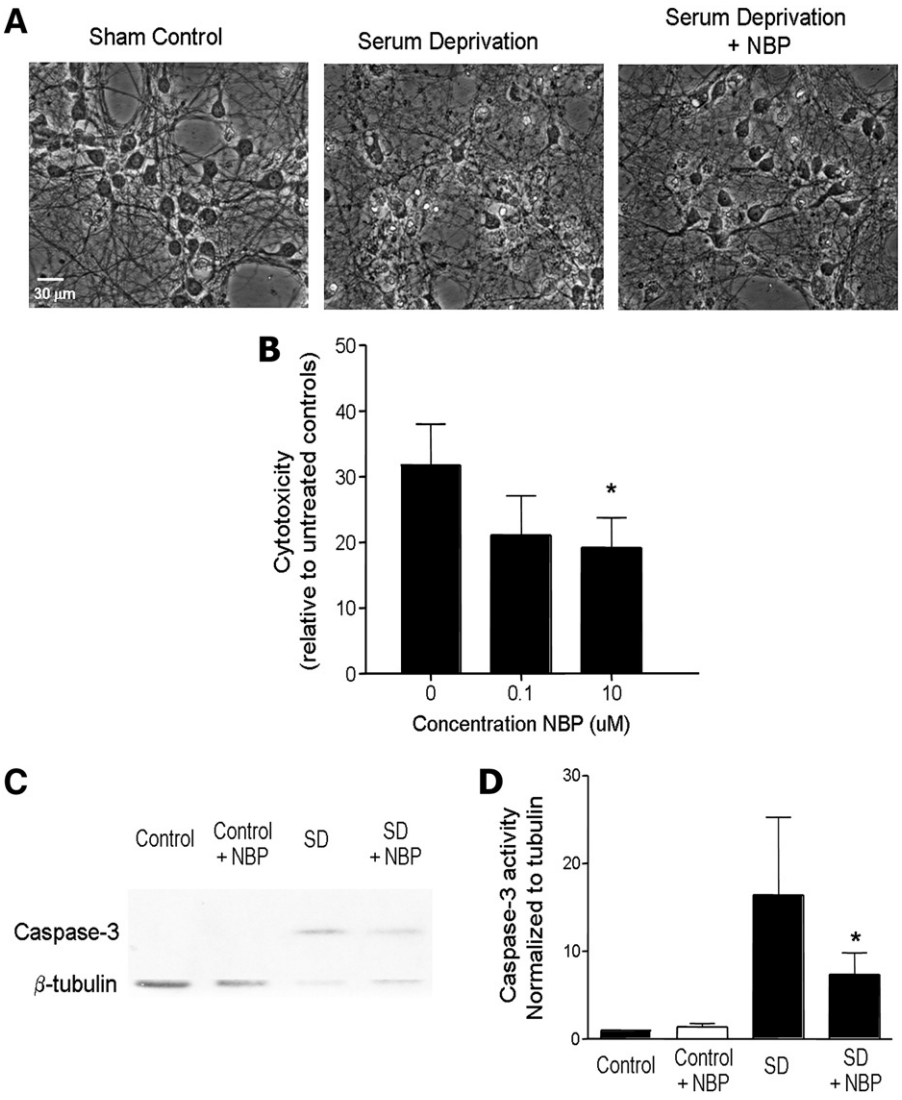

Fig. 1 – Apoptotic cell death and NBP protection in cortical neuronal cultures. Serum deprivation-induced apoptotic cell death was tested in pure neuronal cultures of 7–8 days in vitro. (A and B) NBP (0.1–10 μM) was coapplied to the medium with SD and cell death was assessed using LDH release measurement 72 hrs later. Phase contrast photos in A illustrate conditions of cortical neurons in 72 hrs after sham control, SD, and SD plus 10 μM NBP. The bar graph in B summarizes the toxicity data, showing a significant neuroprotective effect of NBP at 10 μM. N=3 independent assays of different cultures. $^{\#}P<0.05$ compared with controls. (C) Cleaved caspase-3 (17 kDa) was detected using Western blot. SD increased caspase-3 cleavage while it was reduced by coapplied 10 μM NBP. β-Tubulin was used as loading control; the faint bands under SD and SD plus NBP were due to less protein loads. This was corrected in data analysis as shown in normalized density in panel D. (D) Summary of caspase-3 experiments. N=3 independent assays.