

**Review**

# The Neuroprotective Effect and Probable Mechanism of DL-3-n-Butylphthalide in Brain Diseases

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## Key Words

3-n-Butylphthalide · Neuroprotective effect · Parkinson's disease · Alzheimer's disease · Cerebral ischemia

## Abstract

3-n-Butylphthalide (NBP), one of the chemical constituents of celery oil, is used for the treatment of many central nervous system diseases, including Parkinson's disease, Alzheimer's disease and cerebral ischemia. NBP showed potent neuroprotective effects by decreasing oxidative damage, inhibiting inflammatory responses, improving mitochondrial function, and reducing neuronal apoptosis.

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## Introduction of 3-n-Butylphthalide

3-n-Butylphthalide (NBP), an extract from the seeds of *Apium graveolens* Linn (Chinese celery), was synthesized and received approval by the State Food and Drug Administration of China for clinical use in stroke patients in 2002 [1]. NBP is a chiral compound, which contains both L- and D-isomers. Peng et al. [2] found that L-3-n-butylphthalide (L-NBP) attenuated learning and memory deficits induced by chronic cerebral hypoperfusion in rats.

NBP is a new drug which could increase the blood flow of the ischemic area to improve energy metabolism [3], promote microvascular regeneration to reconstruct ischemic microcirculation [4, 5], and protect the mitochondria to rescue ischemic penumbra cells. In addi-

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tion, NBP could reduce the infarct volume, local cerebral ischemia, brain edema and inflammation in animal models [6]. It also has antiplatelet aggregation and antithrombotic effects [7]. Here we provide a brief review of the roles of NBP in Parkinson's disease (PD), Alzheimer's disease (AD) and cerebral ischemia. We also discuss the controversies and challenges in exploiting NBP as a therapeutic strategy.

### The Mechanisms of the Neuroprotective Effect of NBP

The probable mechanisms of NBP may involve: (1) anti-apoptotic effects – NBP plays the role of anti-apoptosis through a mitochondrion-related caspase-dependent or nondependent apoptotic pathway [8–11]; (2) anti-oxidative stress – NBP could inhibit mitochondrial permeability, increase the cellular GSH content, and inhibit the overproduction of nitric oxide to play the role of anti-oxidative stress [1], and (3) improve learning and memory deficits – NBP can reduce abnormal phosphorylation of tau protein by downregulating GSK-3 $\beta$  activity [12] or increase alpha-amyloid precursor protein ( $\alpha$ APP) release by increasing PKC $\alpha$ , PKC $\epsilon$  activity and MAPK phosphorylation levels to reduce  $\beta$ -amyloid protein (A $\beta$ ) generation [13]. Pretreatment with L-NBP could significantly increase cell viability of H<sub>2</sub>O<sub>2</sub>-damaged cells and reduce H<sub>2</sub>O<sub>2</sub>-induced neuronal apoptosis. NBP treatment at a dose of 10  $\mu$ M inhibited H<sub>2</sub>O<sub>2</sub>-induced downregulation of Bcl-2, Bcl-w, and PKC $\alpha$  but also attenuated the overexpression of Bax. The PKC inhibitor calphostin C significantly attenuated the protective effects of NBP. This suggested that NBP might protect neurons against H<sub>2</sub>O<sub>2</sub>-induced apoptosis by modulating apoptosis-related genes and activating the PKC $\alpha$  pathway [9]. Another study reported that vascular endothelial growth factor expression was upregulated, while the caspase-3 expression was reduced in the diabetes mellitus-NBP rats. It showed that NBP may have a protective effect on diabetic brain damage through enhancing vascular endothelial growth factor expression to inhibit caspase-3-mediated apoptosis [10]. In conclusion, NBP is a multi-target clinical drug, suggesting that it may have therapeutic potential for central nervous system diseases.

### The Effect of NBP in PD

The clinical features of PD are resting tremor, muscle rigidity, bradykinesia and postural reflex disorder. Motor symptoms of PD are mainly due to the selective degeneration of substantia nigra dopaminergic neurons in the ventral midbrain, which results in a decrease in dopamine levels in the striatum [14].  $\alpha$ -Synuclein oligomers and aggregates play an important role in the pathogenesis and development process of PD [1, 15]. Protein accumulations in cytoplasmic inclusions are called Lewy bodies. Our previous studies have shown that MPTP-induced oxidative stress promoted the oligomerization of  $\alpha$ -synuclein that leads to the pathological aggregation of  $\alpha$ -synuclein [15].

The present study demonstrated that NBP might be promising for the clinical treatment of patients with PD. NBP can protect against MPP<sup>+</sup>-induced neurotoxicity via activation of an autophagic process [1, 15]. This report strongly suggests that NBP promotes intracellular autophagic activity and subsequently enhances the degradation of  $\alpha$ -synuclein. Thus, it can be concluded that autophagy protects against PD through self-digestion of mis-aggregated proteins, such as  $\alpha$ -synuclein [16]. It has been found that NBP has the ability to suppress the release of cytochrome C, to stimulate the upregulation of vascular endothelial growth factor, and subsequently to protect against oxidative stress in diabetic rats [10, 16].

Similarly, NBP administration reduces oxidative stress in PD models. It does this by accentuating the expression of the vesicular monoamine transporter 2 genes and protecting dopaminergic neuronal tissue by inhibiting oxidative stress [17, 18]. It also ameliorates mitochondrial dysfunction seen in PD [1].

### The Effect of NBP in AD

AD is the most common form of dementia in the elderly and has a variety of clinical symptoms. Its main pathological feature appears to be A $\beta$  deposits in the brain, including senile plaques and neurofibrillary tangles [19]. A $\beta$  is thought to be a key player in neuronal damage and dementia in AD patients. A $\beta$  is a fragment from a larger protein called APP, a transmembrane protein that penetrates through the neuron's membrane. APP is critical to neuron growth, survival and postinjury repair [20].

The mechanism of NBP improving cognitive impairment remains unclear. In a recent study, it was found that NBP ameliorated the impairment of spatial learning and working memory in an A $\beta$ -infused model [12]. The effect may be related to enhanced A $\beta$  clearance and degradation. Moreover, it was reported that NBP increased insulin-degrading enzyme activity in a triple transgenic mouse model carrying human mutant APP, presenilin 1, and tau transgenes [12]. Another report showed that the effect by NBP of lowering cerebral A $\beta$  accumulation may be attributable to directing APP processing toward a nonamyloidogenic pathway [21].

Oxidative damage plays an important role in the development and progression of AD. A $\beta$  directly interacts with mitochondria, and then induces the generation of free radicals, mitochondrial dysfunction and cell death [22]. It was shown that NBP reversed A $\beta$ -induced oxidative injuries. NBP appears to be an effective anti-oxidant. Glutathione peroxidase is one of the main enzymes involved in the cellular protection against damage from oxygen-derived free radicals [12, 23]. Glutathione peroxidase may be an important factor in A $\beta$ -induced oxidative stress. In addition, it may be a target of NBP for preventing A $\beta$ -induced neuronal damage from superoxidation [12].

### The Effect of NBP in Cerebral Ischemia

Cerebral ischemia is induced when there is not enough blood flowing to the brain; in this case, the blood cannot meet the metabolic needs. When the brain is hypoxic, we found that the cerebral metabolism changes and the metabolic rate decreases. This results in the death of brain tissue or ischemic stroke [24].

NBP has the ability to decrease the area of cerebral infarct in focal cerebral ischemic rats [25]. It can also improve energy metabolism in mice with complete brain ischemia [26]. The positive effects of NBP and L-NBP on cerebral ischemia and cerebral infarct have been verified in ischemic patients and animal models; however, little is known about the neuroprotective machinery of NBP.

Mitochondria play a crucial role in apoptosis. NBP also increases the ATP level in rats with cerebral ischemia, and therefore prevents mitochondrial dysfunction resulting from ATP depletion [27]. Xiong and Feng [28] found that NBP improved mitochondrial dysfunction during cerebral ischemia.

## Conclusion

In summary, NBP showed neuroprotective effects by decreasing oxidative damage [29], inhibiting inflammatory responses [6], improving mitochondrial function, and reducing neuronal apoptosis [30]. The neuroprotective effects of NBP have potential therapeutic effects on the treatment of PD, AD and cerebral ischemia patients.

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