DOCTORAL THESIS

Autophagy-inducing Mechanism and Anti-Parkinsonian Effects of a Corynoxine B Analogue CB6

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It affects 7-10 million people worldwide, of which half are in China. PD is characterized by the loss of dopaminergic neurons and the accumulation of abnormal protein aggregates in the midbrain. Unfortunately, current drugs for PD only can replenish dopamine levels, without stopping the disease progression. Therefore, there is an urgent need to develop disease-modifying drugs to prevent the progression of PD. The autophagy–lysosomal pathway (ALP) is a highly conserved process responsible for degrading and recycling cellular contents including protein aggregates and damaged organelles. Accumulating evidence indicates that impairment of ALP plays a critical role in the progression of PD. Thus, enhancing ALP has emerged as a promising strategy for treating PD.

Previously, we identified a natural alkaloid named corynoxine B (Cory B) as a neuronal autophagy inducer. However, the brain permeability of Cory B is relatively low, which limits its potential use in treating PD. In this study, a Cory B derivative with better bioavailability termed CB6 was newly synthesized and its autophagy-enhancing and neuroprotective effects in PD were determined *in vitro* and *in vivo*.

Firstly, transmission electron microscopy showed CB6 increases autophagic compartments. Western blotting and immunofluorescence assays suggested that

CB6 promotes the formation of an autophagy gold marker microtubule-associated protein 1 light chain 3B-II (LC3B-II), indicating CB6 induces autophagy. Although CB6 slightly inhibits the well-established MTORC1 pathway (a major negative regulator of autophagy), CB6-induced autophagy is independent of the MTORC1 pathway. To reveal the regulatory mechanism of CB6, we explored another regulator of autophagy initiation, the PIK3C3 complex. CB6 enhances the activity of the PIK3C3 complex and promotes PI3P production without disturbing the assembly of the PIK3C3 complex. These results indicated that CB6 induces PIK3C3-dependent but MTORC1-independent autophagy.

Secondly, to demonstrate whether CB6 is neuroprotective acting as an autophagy inducer, we investigated the neuroprotective effects of CB6 in a 1-methyl-4-phenylpyridinium (MPP^+)-induced PC12 cell model of PD. CB6 treatment rescued MPP^+-decreased cell viability and the protective roles of CB6 were blocked by autophagy inhibition. In addition, CB6 restored MPP^+-impaired autophagy and alleviated MPP^+-induced apoptosis. Our results demonstrated that CB6 is neuroprotective in the MPP^+-induced cellular model of PD via inducing autophagy.

Finally, in *in vivo* experiments, CB6 has been shown to induce autophagy in the brains of wild-type mice. To evaluate the anti-Parkinsonian effects of CB6 *in vivo*, we used the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. In the MPTP mouse model, CB6 treatment ameliorated motor impairment and attenuated the loss of dopaminergic neurons both in the striatum and substantia

nigra pars compacta (SNpc) of mouse brains. Moreover, CB6 treatment rescued the decrease in striatal dopamine and its metabolites DOPAC and HVA levels. In summary, CB6 has neuroprotective effects on dopaminergic neurons in the MPTP mouse model of PD.

Collectively, the small molecular compound CB6 is a brain-permeable autophagy enhancer via PIK3C3 complex activation, making it an orally effective drug candidate for treating PD.