ABSTRACT

Parkinson’s disease (PD) is the second most common neurodegenerative disorder characterized by the accumulation of protein aggregates (namely Lewy bodies) in dopaminergic neurons in the substantia nigra region of the brain. Alpha-synuclein (α-syn) is the major component of Lewy bodies (LBs) in PD, and impairment of the autophagy-lysosomal pathway has been linked to its accumulation. In our previous study, we identified corynoxine B (Cory B), an oxindole alkaloid isolated from Uncaria rhynchophylla (Miq.) Jacks (Gouteng in Chinese), as a Beclin-1-dependent autophagy enhancer. In this work, we continued to screen autophagy enhancers from Gouteng alkaloids, and found corynoxine (Cory), an isomer of Cory B, also induces autophagy in different neuronal cell lines and primary neurons. Meanwhile, Cory promotes the formation of autophagosomes in the fat bodies of Drosophila. By inducing autophagy, Cory promotes the clearance of wild-type and A53T α-syn in inducible PC12 cells. Interestingly, different from its enantiomer Cory B, Cory induces autophagy through the Akt/mTOR pathway as evidenced by the reduced levels of phospho-TSC2, phospho-Akt, phospho-mTOR and phospho-p70 S6 Kinase.

To identify the different pathway between Cory and Cory B, we performed phosphoproteomic study on N2a cells. With the help of iGPS (In vivo Group-based Prediction System), protein kinases which were significantly regulated by Cory or
Cory B were predicted. Based on these kinases, we drew the detailed kinase-substrates network regulated by Cory or Cory B. The structures of Cory and Cory B differ only in the stereochemistry at the spiro carbon; however, Cory has more effect on the CAMK, Trb and TSSK families, while CDK and CDKL families are more sensitive to Cory B.

Furthermore, we established a rotenone rat model of PD via injecting rotenone into the substantia nigra pars compacta (SNC) and ventral tegmental area (VTA), and evaluated the neuroprotection of Cory and Cory B on this rat model. Motor dysfunction, decreased TH level, impairment of autophagy, aggregation of α-syn and activation of microglia were all found on this PD model, which were consistent with previous reports. After the treatment of Cory or Cory B, we found that both Cory and Cory B improve motor dysfunction, increase the TH level, and inhibit microglial activation. Both Cory and Cory B decrease the puncta number of aggregated α-syn, likely due to the induction of autophagy. All these results indicate the neuroprotection of Cory and Cory B against PD.

Collectively, our findings (1) provide the original finding of Coy to be an autophagy enhancer with experimental evidences that Cory inhibited the pathway of Akt/mTOR; (2) provide cellular and animal experimental evidences for developing Cory or Cory
B as anti-PD agent, by inducing autophagy in neurons; and (3) provide candidate pathways to identify the primary molecular target of Cory or Cory B, which may turn out to be potential therapeutic targets for treating PD.

**Keywords:** Parkinson’s disease, Cory, Cory B, autophagy, phosphoproteomic, neuroprotection
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