DOCTORAL THESIS

Protopine and Its Derivatives Mitigate Tau Pathology Via Histone Deacetylase 6 Inhibition in Alzheimer's Disease Models

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ABSTRACT

The low success rates of clinical trials for Alzheimer's Disease (AD) medicament has demanded for safer and effective novel drugs. In AD, neurofibrillary tangles (NFTs) enriched with hyperphosphorylated tau is the causative factor for memory and cognitive imbalance. Emerging evidence has demonstrated an impairment of the ubiquitin-proteasomal system (UPS) upon accumulation of hyperphosphorylated tau. Histone deacetylase 6 (HDAC6) is a vital enzyme involved in tau hyperphosphorylation and cognitive dysfunction in AD animal models. Furthermore, previous studies have shown that downregulating HDAC6 activity and enhancing carboxy-terminus of HSC70 interacting protein (CHIP) mitigates tau pathology in AD. In the current study, we have elucidated the cognition-enhancing effects and the underlying mechanisms of isoquinoline alkaloid, Protopine (PRO), isolated from traditional Chinese medicine, *Corydalis yanhusuo.* PRO specifically reduced the hyperphosphorylated tau and alleviated the learning and memory dysfunction in triple transgenic 3xTg-AD mice and mutant tau overexpressed P301S tau mice models. Molecular docking, solid-phase binding, and fluorometric assays suggested that PRO directly binds to the catalytic domain 1 (CD1) of HDAC6 and inhibits its activity. PRO enhanced the expression of molecular chaperones and CHIP E3 ubiquitin ligase *in vitro* and *in vivo*, which further promoted the degradation of ubiquitinated tau via the ubiquitin-proteasomal system (UPS).

To develop more potent HDAC6 inhibitors with optimized brain bioavailability for treating AD, a chemical derivative of PRO, termed PRO-Br, was synthesized. The physicochemical charecteristics were evaluated by Nuclear magnetic resonance spectroscopy and tandem mass spectrometry. In 3xTg-AD and P301S mice models, PRO-Br specifically promoted the

degradation of sarkosyl insoluble tau. Additionally, immunohistochemical results demonstrated that PRO-Br significantly reduced the hyperphosphosphorylated tau in both AD model mice. The fluorometric assay showed that PRO-Br decreased HDAC6 activity in a dose-dependent manner.

Altogether, our findings demonstrated that 1) PRO-Br is a better blood-brain barrier permeable compound than PRO; 2) PRO-Br is a better inhibitor of HDAC6 activity, compared to PRO; 3) PRO and PRO-Br can enhance the expression of molecular chaperones and specifically promote the degradation of pathological hyperphosphorylated tau in 3xTg-AD as well as P301S tau AD models; 4) PRO and PRO-Br can mitigate the memory and cognitive impairment in both AD model mice; and 5) PRO and PRO-Br can be developed into potential drugs for treating AD.

Keywords: Alzheimer's disease, Phospho-tau, Protopine, PRO-Br, 3xTg-AD mice, P301S tau mice, HDAC6.

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