DOCTORAL THESIS

Study of a novel curcumin-derived TFEB activator C1 on experimental alzheimer's disease

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ABSTRACT

Autophagy is the major cellular, conservative, lysosomal catabolic process to eliminate and recycle intracellular waste and organelles through autophagosomes. Enhancing autophagy to promote the clearance of toxic proteins is developing as a promising approach to treat proteinopathy disorders like Alzheimer's disease (AD). AD is the most common aging-associated neurodegenerative disease. It is characterized by the aggregation of aberrantly hyperphosphorylated tau (p-Tau) and excessively produced Amyloid-beta (Aβ) into neurofibrillary tangles (NFTs), and amyloid plaques (AP) respectively. Reprogramming autophagy lysosomal pathway (ALP) through autophagy master controller, transcription factor EB (TFEB), is developing as an attractive strategy to treat AD. It is already proven that TFEB overexpression can promote Aβ and p-Tau lysosomal clearance, attenuate NFT and AP deposition and restore the behavioural deficits in AD mice models. Previously Song et al., 2016 have identified a small molecule curcumin derivative C1. They reported that C1 could bind to recombinant TFEB and enhance ALP both *in vitro* and *in vivo* conditions independent of mTOR inhibition. In the current study, C1 is systematically evaluated for its bioavailability, anti-AD efficacy *in vitro*, and *in vivo* AD experimental models.

To validate TFEB mediated anti-AD efficacy of C1 *in vitro*, we tested the C1 effect on amyloid precursor protein (APP) and p-Tau degradation *in vitro* neuronal AD cell culture models. In N2a cells overexpressed with APP (695) and EGFP-*P301L* tau plasmids, C1 induced APP, CTFβ, and Tau lysosomal degradation. To demonstrate the TFEB dependent autophagic clearance effects of C1, TFEB is silenced in N2a cells with lentiviral shRNA

particles. Under TFEB silenced condition, C1 induced reduction of FL-APP, CTFβ, and Tau was compromised. Overall *In vitro* experiments show that C1 induced lysosomal digestion of FL-APP, CTFβ, and p-Tau in a TFEB dependent manner.

To further demonstrate C1 brain bioavailability, C1 and curcumin comparative pharmacokinetic studies (Pk study) in both mice (time course Pk study) and rat (single time point analysis) are conducted. In Pk studies, both C1 and curcumin are dosed at 10 mg/kg to determine their concentration in the whole brain (mice), separate brain regions (rat), CSF (rat), and plasma. The WinNonlin analysis of C1 and curcumin mice Pk study data revealed that C1 is significantly more bioavailable than curcumin in both brain and plasma, which is also corroborated by the single time point analysis in rats. To illustrate C1 anti-AD activity *in vivo*, C1 is screened in homozygous P301S (Tau), heterozygous 5xFAD (Aβ), and homozygous 3xTg (both Aβ and Tau) AD transgenic mice models. These mice were started to treat with C1 before the onset of AD pathology until the AD pathological phenotype is expressed to cause impairment in mice behaviour. In mice behavioural examination, C1 treatment has significantly improved mice motor function (Rotarod-P301S), restored cognitive impairment related to the cortex (contextual fear conditioning-5xFAD), hippocampus (Morris water maze-3xTg) and improved cholinergic activation (open field-3xTg). In the brain biochemical examination, C1 activated the TFEB mediated ALP pathway to degrade FL-APP, CTFα/β, Aβ, and p-Tau and reduced the amyloid plaque load, extra neuronal-NFT

positive cells. Notably, C1 treatment in 5xFAD mice has significantly restored hippocampal synaptic function.

In summary, the current study validates C1 as an orally bio-available potent small molecule TFEB activator which restores mice cognitive impairment, altered behaviour, and synaptic plasticity by reducing Aβ and tau levels in AD experimental models. Overall, the TFEB activator C1 can be a promising drug to treat AD.

Key words: Alzheimer's disease, Curcumin derivative C1, Autophagy, TFEB.

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