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Effects of Curcumin on the Expression of Flotillin-1 in the Brains of APP/PS1 Double Transgenic Mice

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Abstract

Alzheimer's Disease (AD) is a chronic and progressive neurodegenerative disease. Flotillin-1 is a marker protein of lipid raft in nervous tissue, which is significantly increased in AD. To investigate the changes of flotillin-1 expression in the brains of APP/PS1 double transgenic mice treated with curcumin, thirty six-month-old APP/PS1 double transgenic mice were randomly divided into model group (MOD), low-dose curcumin group (LCUR) and high-dose curcumin group (HCUR). LCUR group mice and HCUR group mice were fed with curcumin (0.16 $g \cdot kg^{-1}$ and 1.0 $g \cdot kg^{-1}$, respectively) every day for 6 months. Western blot and real-time PCR were used to detect the expression of flotillin-1 in brain. Results showed that compared with MOD group, the expression of flotillin-1 protein ($P < 0.05$) and mRNA ($P < 0.01$) in brains of HCUR group were statistically decreased. Thus, high-dose curcumin may play a neuroprotective role in AD through downregulating the expression of flotillin-1 in APP/PS1 double transgenic mice.

Keywords

Alzheimer's Disease, Curcumin, Flotillin-1, Lipid Raft

1. Introduction

Alzheimer's Disease (AD) is a neurodegenerative pathology first described by Aloïs Alzheimer in 1907 as an "unusual illness of the cerebral cortex" [1]. AD is a chronic and progressive neurodegenerative disease with nervous tissue atrophy, neuron and synapse loss, and neuronal subcellular structure and function disorders. The manifestations of AD in clinic include gradual memory loss, alte-

ration of the individual's personality and failure to communicate or perform routine tasks. Flotillin-1 is a marker protein of lipid raft in nervous tissue, which is significantly increased in AD nerve cells. It was reported that flotillin-1 accumulated in lysosomes of tangle bearing neurones in the course of AD [2]. Flotillins 1 and 2 increased in pyramidal neurons and astrocytes in the white matter during the formation of senile plaque in Alzheimer's disease [3]. These findings suggest that flotillins are associated with progression of Alzheimer pathology. Curcumin possesses anti-carcinogenic, anti-oxidant and anti-inflammatory properties and may help delay or prevent neurodegenerative diseases, including AD [4]. In this study, flotillin-1expression levels were studied in curcumin treated APPswe/PSEN1dE9 (APP/PS1) transgenic mice to explore the mechanism of curcumin in AD prevention and treatment.

2. Materials and Methods

1) Animals APP/PS1 double transgenic mice (B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J) were purchased from the Institute of Model Animal of Nanjing University (Animal license: No. SCXK (Su) 2016-0012). Thirty 6-month-old APP/PS1 double transgenic mice were randomly divided into model group (MOD), low-dose curcumin group (LCUR) and high-dose curcumin group (HCUR)，ten mice in each group. Mice in LCUR group and HCUR group were fed with 0.16 $g \cdot kg^{-1}$ and 1.0 $g \cdot kg^{-1}$ curcumin respectively for 6 months. All animals were housed in the Laboratory Animal Center of Chongqing Medical University (Animal license: No. SCXK 07-0001). They were cultivated in a temperature and humidity-controlled (24˚C, 40% - 70%) pathogen-free vivarium, on a 12:12-hour light-dark cycle (12-hour light, 12-hour dark) with free access to food and water. Their use was approved by the Animal Ethics Committee of Chongqing Medical University.

2) Reagents Curcumin was purchased from Sigma-Aldrich (Bangalore, India). RNAiso Plus, PrimeSript™ RT reagent Kit and SYBR Premix Ex Taq™ II Kit were purchased from TaKaRa (Dalian, China). Flotillin-1 and β-actin primer were designed and synthesised by Shanghai Shenggong Bioengineering co. LTD. Monoclonal anti-flotillin-1 antibody was purchased from BD Biosciences (United States), monoclonal anti-β-actin monoclone antibody was purchased from Sigma-Aldrich (Bangalore, India).

3) Western blot Total protein samples from each group of brain tissue were lysed with RIPA lysis buffer following the manufacturer's instructions. Protein concentrations were measured using the BCA method. A total of 20 μg proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Nonspecific binding was blocked with 5% skimmed milk at room temperature for 1 h, the membranes were incubated with primary antibody (flotillin-1, 1:500; β-actin, 1:500) at 4˚C overnight. The next day, the membranes were washed three times with 0.1% Tween-20/TBST (pH 7.6) and incubated with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibodies

for 2 h at room temperature. An enhanced chemiluminescence detection reagent was used for enhanced blot detection. The band intensity was assessed with Image Lab Software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and analyzed.

4) Quantitative real-time PCR Total RNA was extracted from the brain tissues by RNAios Plus reagent according to the manufacturer's instructions. RNA samples from each group were reverse transcribed into cDNA using PrimeScript™ RT reagent Kit. Quantitative RT-qPCR was performed on a Light Cycler thermal cycler system (Bio-Rad, United States) using SYBR®Premix Ex Taq™II. Primers are listed as follows: flotillin-1 forward
5'-GCCGAGTGTTTGTCCTACC-3', reverse
5'-AATGCCCGTGACTGAGATG-3'; *β*-actin forward
5'-ATATCGCTGCGCTGGTCGTC-3', reverse:
5'-AGGATGGCGTGAGGGAGAGC-3'.

5) Statistical Analysis All statistical analyses were performed using SPSS software (version 18.0) and values are expressed as means ± standard deviation (mean ± SD). Differences between multiple groups were analyzed by One-way analysis of variance (ANOVA). Pairwise comparison was analyzed by SNK-q test. *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Curcumin Downregulated the Expression of Flotillin-1 in APP/PS1 Transgenic Mice

In order to assess the expression of flotillin-1, western blot was performed and the relative level of flotillin-1 expression was calculated as the ratio to the internal control. The results showed that the expression of flotillin-1 downregulated in 1.0 $g \cdot kg^{-1}$ curcumin treated APP/PS1 transgenic mice, while there were no significant changes in 0.16 $g \cdot kg^{-1}$ curcumin treated mice (**Figure 1**).

3.2. Curcumin Inhibited the mRNA Leved of Flotillin-1 in APP/PS1 Transgenic Mice

In addition, flotillin-1 expression was also confirmed by Real-time PCR. The results showed that mRNA level of flotillin-1 decreased in 1.0 $g \cdot kg^{-1}$ curcumin treated APP/PS1 transgenic mice ($P < 0.01$), while there were no significant changes in 0.16 $g \cdot kg^{-1}$ curcumin treated mice (**Figure 2**).

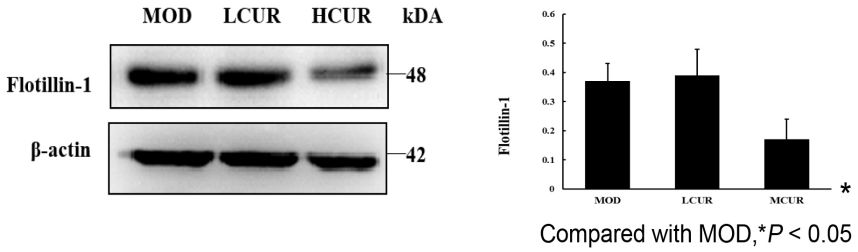

Figure 1. Expression of flotillin-1 protein in brains of APP/PS1 double transgenic mice (n = 3, $\bar{x}$ ± s).

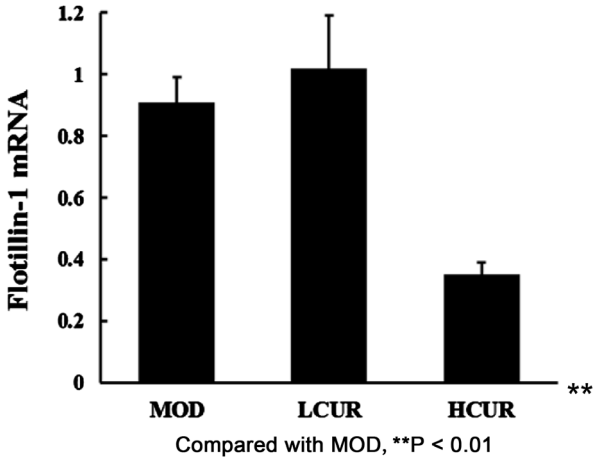

Figure 2. Expression of flotillin-1 mRNA in brains of APP/PS1 double transgenic mice (n = 3, $\bar{x}$ ± s).

4. Discussion

Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. The etiology of AD is not entirely clear and there are no therapeutic interventions that can delay the disease progression.

Lipid rafts are membrane microdomains that are enriched in cholesterol and sphingolipids [5]. Lipid rafts have the ability to cluster to form larger, more-ordered platform within the liquid-disordered bilayer of cellular membranes. They involved in cellular processes such as protein trafficking and signaling processes, and which play a fundamental role in membrane fluidity and budding. At present, caveolin protein, flotillin protein, G-protein, GPI-anchored protein, receptor tyrosine kinase and non-receptor tyrosine kinase are known to be abundant in lipid rafts.

Flotillin is a biochemical marker of lipid rafts and has been shown to be associated with cell signaling, regulation of cell-cell contacts, membrane-cytoskeletal interactions, and endocytosis. The family of flotillin proteins contains two well-conserved, ubiquitously expressed members, flotillin-1 and flotillin-2. Flotillin-1 is expressed mostly in brain, lung, placenta and hematopoietic cells [6] [7]. It could be ultrastructural raft markers in neural tissue. Flotillin-1 was involved in cellular signal transduction, endocytosis and cell adhesion. Flotillins are associated with progression of Alzheimer pathology. Kokubo H. *et al.* found that flotillins were abundant in pyramidal neurons and astrocytes in the white matter [8]. Flotillins became stronger with the development of senile plaque formation and strongest in Alzheimer's disease. Ehehalt *et al.* reported that flotillin-1, as a specific lipid rafts protein, could recruit amyloid precursor protein (APP) into lipid rafts, thus providing a platform for cleavage of APP to release amyloidogenic amyloid beta (Aβ) peptide [9]. The enhanced expression of flotillin-1 in brain tissue of AD patients was positively correlated with the Aβ levels. Chen TY *et al.* reported that flotillin-1 interacted with APP intracellular domain (AICD) and may recruit APP to lipid rafts, therefore participated in the localiza-

tion and processing of APP [10]. Hattori *et al.* found that in BACE1-HEK cells, overexpressed flotillin-1 could recruit β-site APP cleaving enzyme-1 (BACE1) into lipid rafts and regulate the function of BACE [11]. Nishikawa *et al.* also detected the abnormally enhanced expression of flotillin-1 in NFTs, suggesting that flotillin-1 may be involved in the pathological changes of tau/NFTs in AD [12].

Curcumin is a chemical component extracted from the rhizome of turmeric. In recent years, curcumin has been reported to possess anti-amyloidogenic, anti-inflammatory, anti-oxidative, and metal chelating properties that may result in potential neuroprotective effects, but the mechanism is still not clear. In this study, we observed that the protein and gene expression of flotillin-1 decreased obviously in 1.0 $g \cdot kg^{-1}$ treated APP/PS1 double transgenic mice. While in 0.6 $g \cdot kg^{-1}$ treated APP/PS1 double transgenic mice, there were no significant changes of flotillin-1. It indicated that the neuroprotective effect of curcumin may be relevant with regulation of flotillin-1 in a certain concentration.

At present, several studies have reported that Chinese medicine ingredients and the combination of traditional Chinese and western medicine may play neuroprotective roles through regulation of flotillin-1. Zhu H. *et al.* reported that Yizhijia*nnao* granule could regulate the expression of various proteins include flotillin-1 in the temporal lobe of senescence-accelerated mouse prone 8 mice, and might be therapeutically beneficial for the treatment of Alzheimer's disease [13]. Lu Z. *et al.* found that curcumin protected cortical neurons against oxygen and glucose deprivation/reoxygenation injury through flotillin-1 and extracellular signal-regulated kinase 1/2 pathway [14].

Flotillins have a role in a large number of physiopathological processes, mainly through their function in regulating axon regeneration, neuronal differentiation, endocytosis, T cell activation, insulin signaling, membrane protein recruitment and so on. Our study found that high-dose curcumin may play a neuroprotective role in AD through downregulating the expression of flotillin-1 in APP/PS1 double transgenic mice, and the mechanisms need to be further studied.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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