Curcumin ameliorates lipid metabolic disorder and cognitive dysfunction via the ABCA1 transmembrane transport system in APP/PS1 double transgenic mice

Mingyuan Tian[1,2,†], Fanlin Zhou[2,3,†], Zhipeng Teng[2], Chen Wang[2], Xiong Zhang[2], Yangyang Wang[3], Yu Li[2,3,*]

[1] Department of Endocrinology, The Second Affiliated Hospital of Chongqing Medical University, 400010 Chongqing, China

[2] Institute of Neuroscience, School of Basic Medicine, Chongqing Medical University, 400016 Chongqing, China

[3] Department of Pathology, Chongqing University Cancer Hospital & Chongqing Cancer Institute & Chongqing Cancer Hospital, 400030 Chongqing, China

*Correspondence: liyu100@cqu.edu.cn (Yu Li)
[†] These authors contributed equally.

The disorder of lipid metabolism, especially cholesterol metabolism, can promote Alzheimer's Disease. Curcumin can ameliorate lipid metabolic disorder in the brain of Alzheimer's Disease patients, while the mechanism is not clear. APP/PS1 (APPswe/PSEN1dE9) double transgenic mice were divided into dementia, low-dose, and high-dose groups and then fed for six months with different dietary concentrations of curcumin. Morris water maze was used to evaluate the transgenic mice's special cognitive and memory ability in each group. In contrast, the cholesterol oxidase-colorimetric method was used to measure total serum cholesterol and high-density lipoprotein levels. Immunohistochemistry was used to evaluate the expression of liver X receptor-β, ATP binding cassette A1 and apolipoprotein A1 of the hippocampus and Aβ42 in the brains of transgenic mice. The mRNA and protein expression levels of liver X receptor-β, retinoid X receptor-α and ATP binding cassette A1 were evaluated using qRT-PCR and Western blotting, respectively. Curcumin improved the special cognitive and memory ability of transgenic Alzheimer's Disease Mice. The total serum cholesterol decreased in Alzheimer's Disease mice fed the curcumin diet, while the high-density lipoprotein increased. The curcumin diet was associated with reduced expression of Aβ and increased expression of liver X receptor-β, ATP binding cassette A1, and apolipoprotein A1 in the CA1 region of the hippocampus. The mRNA and protein levels of retinoid X receptor-α, liver X receptor-β, and ATP binding cassette A1 were higher in the brains of Alzheimer's Disease mice fed the curcumin diet. Our results point to the mechanism by which curcumin improves lipid metabolic disorders in Alzheimer's Disease via the ATP binding cassette A1 transmembrane transport system.

Keywords

1. Introduction

Alzheimer's disease (AD) is an insidious neurodegenerative disorder. The clinical features of AD are progressive cognitive dysfunction and language and behavioral disorders, social dysfunction, and eventually death [1]. Cholesterol is an essential part of cell membranes and myelinated axons, thereby playing an essential role in maintaining neuronal integrity and functionality [2]. Although the brain is the organ with the highest cholesterol content in humans [3], it cannot absorb cholesterol directly from the blood because of the blood-brain barrier. Cholesterol synthesis in the brain is therefore carried out mainly by astrocytes and neurons [4]. Cholesterol metabolism disorder is a major risk element to cardiovascular disease and may play a role in AD. Several clinical and epidemiological studies have reported a close link between cholesterol metabolism abnormality and AD [5].

ABCA1, a member of the ATP-binding cassette family, plays a pivotal role in cholesterol transport. The cholesterol transport regulated by ABCA1 also plays an essential part in the formation of β-amyloid ($A\beta$) deposits [6]. ABCA1 is a recently discovered important transmembrane protein that can mediate reverse cholesterol transport and promote the cellular efflux of cholesterol to various acceptors, including HDL (High-density lipoprotein) [7]. Under normal circumstances, the cholesterol transported by ABCA1 into the extracellular fluid can combine with apolipoprotein A1 (apoA1) to form β-HDL. β-HDL develops further into mature HDL, which can then help to reverse cholesterol transport [8]. ABCA1 in the body is mainly regulated by the liver X (LXR) and retinoid X (RXR) receptors. A recent study found the ABCA1 transmembrane transport system mediated by LXR and RXR played an essential role in cholesterol balance [9]. ABCA1 also participates in $A\beta$ transport, deposition and senile plaque formation [2].

Curcumin is a special phenolic compound derived from the plant Curcuma longa. It is used clinically as an antioxidant, anti-inflammatory, anti-atherosclerosis, anti-coagulation and anti-tumor agent [10]. The role of curcumin in reducing cholesterol levels is attracting more and more attention. Many researchers have started using cur-

cumin to treat various transgenic animal models for diseases caused by lipid metabolism disorders, such as arteriosclerosis and hypercholesterolemia, with positive results. These studies demonstrated that curcumin could reduce cholesterol levels to maintain body balance [11, 12]. However, the brain cholesterol level is not correlated with the blood cholesterol concentration, and its synthesis mechanism is also different [13]. It is unknown whether curcumin can reduce age-related changes in the brain and gradually increase cholesterol levels in the neurons of AD patients.

This research uses the APP/PS1 transgenic mouse model to observe the impact of different curcumin concentrations on cholesterol content and the LXR/RXR-mediated ABCA1 transmembrane transport system. This original experimental evidence should help to find new treatments and targets for AD.

2. Materials and methods

2.1 Animals

APP/PSI (APPswe/PSEN1dE9) transgenic mice (Alzheimer's disease mouse model) and the type of wild C57BL/6J mice (control mice) were supplied through the Institute for Laboratory Animals, Nanjing Biomedical Research Institute, Nanjing University. Total animal experiments were carried out by the guidelines to Care and Use for experimental Laboratory Animals (NIH Publication No. 85–23, revised 1996). They were accepted through the Research Ethics Committee of the Chongqing University Cancer Hospital. Six-month-old APP/PSI double transgenic mice were divided into three groups randomly. They fed different concentrations of curcumin-mixed feeds: the control group was fed a regular diet without curcumin. The Cur-L group was fed 0.16 g/kg of curcumin- (Sigma-Aldrich) mixed, and Cur-H was fed 1.0 g/kg of curcumin-mixed feeds. The experiments were carried out after feeding this diet for six months.

2.2 Tissue preparation

Mice were anesthetized by isoflurane deeply before obtaining tissue samples. A proportion of the mice was infused with 0.9% normal saline, and the brain tissues were stored at –80 °C. The remaining mice were infused with 0.9% normal saline containing 4% paraformaldehyde (PFA) solution.

2.3 Antibodies

Mouse anti-ABCA1 and goat anti-LXR-β antibodies were purchased through Abcam. Rabbit anti-RXR-α, mouse anti-apoA1 and rabbit anti-β-actin were purchased through Santa Cruz. While secondary antibodies were purchased through Bioworld Technology.

2.4 Morris water maze (MWM) test

Specific protocols for the Morris water maze test were described in our previous research [14]. The experiment included a visual platform test (day 1), a hidden platform test (days 2 to 6), and a probe test (day 7).

2.5 Measurement of serum total cholesterol (TC) and HDL

Liquids were mixed according to instructions for the total cholesterol (CHO enzymatic) test kit and the serum high-density lipoprotein kit (Nanjing Jiancheng Biological Engineering). Samples were then added according to the relevant instructions, and the absorbance was measured for each tube.

2.6 Western blot assay

The specific protocol for Western blot assay is described previously [14]. Protein from brain tissues was homogenized using a protein extraction reagent (RIPA:PMSF = 100:1). Protein concentrations were identified through the BCA assay (Beyotime, China). For Western blot analyses, proteins (35 μg) were separated through SDS-PAGE (8% or 10% gels) and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, MS). After blocking, the membranes were incubated overnight at 4 °C with primary antibodies. After washing, the bound antibodies were detected by incubation for 1–2 h at room temperature with secondary antibodies. The membranes were then developed using a Commercial enhanced chemiluminescence system (ECL; Bio-rad), and quantified the signal using Quantity One image analysis (Bio-RAD, USA).

2.7 Immunohistochemistry

The brains of mice were fixed with 4% paraformaldehyde, embedded in paraffin, sliced five μm thick, dewaxed with xylene and ethanol. After washing with TBS, Horseradish peroxidase (HRP) stained slides were treated with 0.3% H_2O_2 for 30 min to inactivate endogenous peroxidase. Then the portions were blocked by 20% goat serum in TBS for one h. LXR-β, RXR-α, ABCA, and apoA1 were detected with the corresponding antibodies, HRP-labeled avidin-biotin complex, and DAB substrate. Specimens were then observed using a fluorescence microscope (LEICA).

2.8 The analysis of quantitative real-time PCR (qRT-PCR)

RNA was separated through brain tissues using RNAiso Plus (TaKaRa). PrimeSriptTM RT reagent Kit (TaKaRa) compound first-strand cDNA based on the manufacturer's introduction. The cDNA was further amplified by SYBR Premix Ex TaqTM II (TaKaRa) in a 20 μL reaction mix using the following primers: ABCA1-F: 5′-CAATGTCAAGGTGTGGTTCAAT-3′; ABCA1-R: 5′-GCTGCTGTTTAGTGAGGTTCAA-3′; RXR-α-F: 5′-TACGCAAAGACCTGACCTACAC-3′; RXR-α-R: 5′-ACTCCACCTCGTTCTCATTCC-3′; LXR-β-F: 5′-TAGAAACAGCCAGACGCTACAA-3′; LXR-β-R: 5′-GGCGATAAGCAAGGCATACTCT-3′; β-actin-F: 5′-TCCTGTGGCATCCACGAAACT-3′; β-actin-R: 5′-GAAGCATTTGCGGTGGACGAT-3′.

2.9 Statistical analysis

Statistical analyses were performed using SPSS software (version 17.0, IBM Corp., Chicago, IL, USA). Experimental data are represented by the mean and standard error of the mean (SEM). Experimental results were compared using
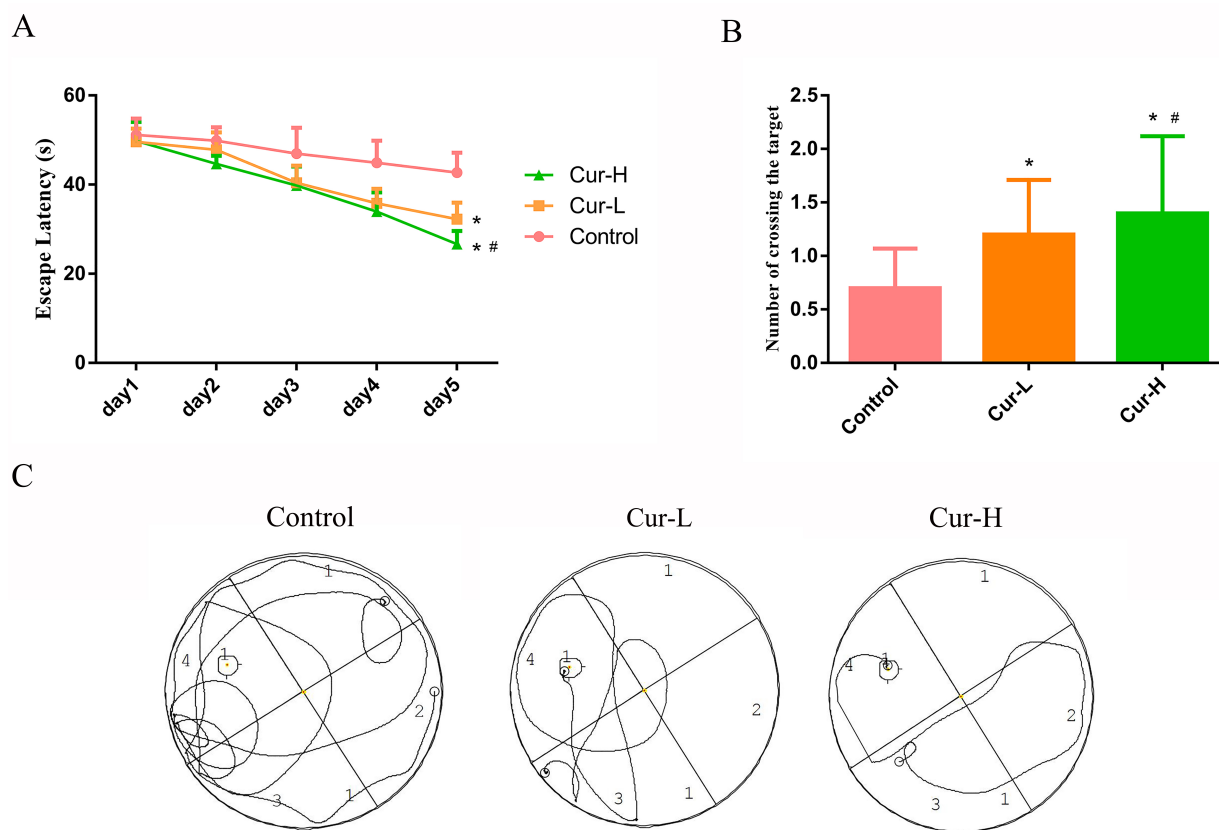
Fig. 1. Curcumin improved cognitive function of APP/PS1 double transgenic mice. (A) The escape latency during the 5-day MWM testes. (B) The number of crossing the target on the last day of the MWM test (n = 5, two-way repeated-measures ANOVA, Tukey's test, #$p < 0.05$, versus Cur-L; *$p < 0.01$, versus Control). (C) The representative route track map for every group to mice.

two-way ANOVA, next by multiple comparisons using Bonferroni post-test. $p < 0.05$ was considered to show statistical significance.

3. Results

3.1 Curcumin improved cognitive function of APP/PS1 mice

MWM was used to evaluate the hippocampal-dependent spatial learning and memory ability of the APP/PS1 double transgenic mouse model to AD. During the learning phase (Fig. 1A), the mice fed low-dose Curcumin (Cur-L group) demonstrated significantly shorter times to discover the evacuation platform (shorter escape latencies) compared to mice from the control group ($p < 0.01$). The average escape latency was also shortened in mice fed high-dose Curcumin (Cur-H group) ($p < 0.01$). In the probe test of spatial memory retention (Fig. 1B), the number of passes through the previous platform location was significantly reduced in the CUR-L group compared to the control group. The platform crossing frequency decreased even further in mice fed the higher curcumin dose ($p < 0.01$). Representative swim paths are displayed in Fig. 1C.

3.2 Curcumin reduced Aβ in the hippocampus of APP/PS1 mice

Immunohistochemistry was used to detect Aβ plaque in APP/PS1 mice (Fig. 2). These plaques were extensive, mostly

round, radiating, with a deep core in the control group. The fraction of occupied area and the number of amyloid protein plaques was lower in the Cur-L group (both $p < 0.05$) than controls, and even more so in mice fed high-dose curcumin (both $p < 0.01$).

3.3 Curcumin reduced the level of TC and increased the level of HDL in the serum of APP/PS1 mice

Using enzymatic detection, the serum TC levels for each group were found to be different (Fig. 3). Serum TC levels in the Cur-L group were dramatically lower than in the control group ($p < 0.05$), even more so in the Cur-H group ($p < 0.05$). However, there was no difference significantly in the serum TC level between the low- and high-dose curcumin groups ($p > 0.05$). Colorimetric detection showed the serum HDL levels of each group were also notably different (Fig. 3); The serum HDL levels in both the Cur-L and Cur-H groups were significantly higher than those in the control group ($p < 0.05$).

3.4 Curcumin promoted the expression of ABCA1 and apoA1 in the hippocampus of APP/PS1 mice

Western blotting and immunocytochemistry were used to detect the ABCA1 expression and apoA1 in each group. Low-dose curcumin treatment enhanced the expression of ABCA1 and apoA1 in the hippocampus of APP/PS1 double trans-
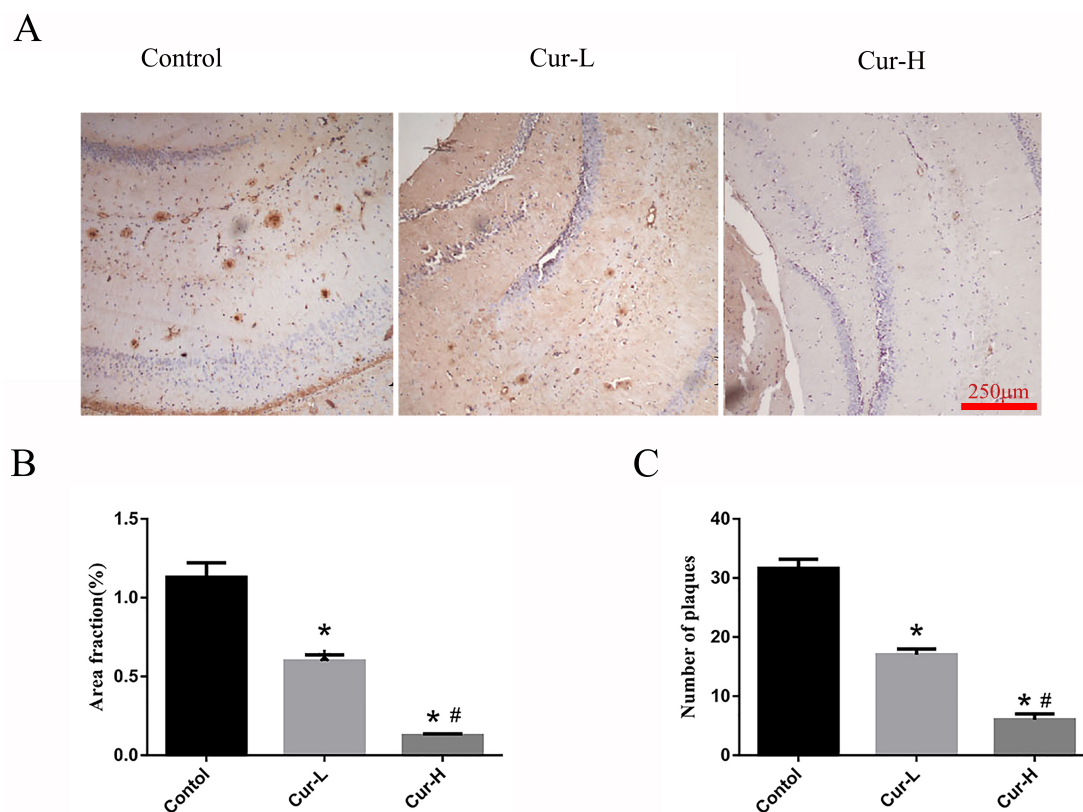
Fig. 2. Curcumin reduced Aβ in the hippocampus of APP/PS1 mice. (A) Immunohistochemical staining displays amyloid protein was expressed in the hippocampus of different groups (Scale bar = 250 μm). (B,C) Area fraction and number of plaques of Aβ in every group (#$p < 0.05$, versus Cur-L; *$p < 0.01$, versus Control).
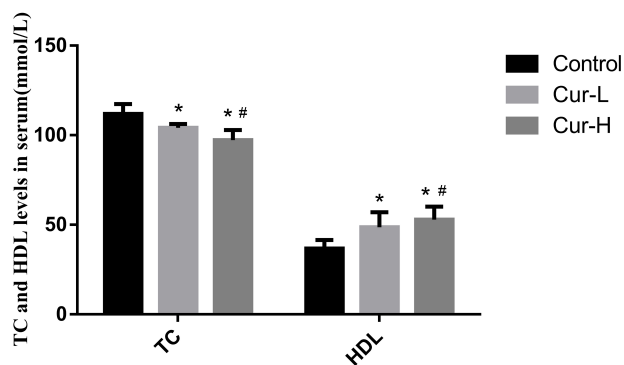

Fig. 3. Curcumin reduced the level of TC and increased HDL in the serum of APP/PS1 mice (#$p > 0.05$, versus Cur-L; *$p < 0.05$, versus Control).

genic mice (both $p < 0.05$). High-dose Curcumin promoted the expression even more, as indicated by the increased intensity of immunocytochemical staining (Fig. 4).

3.5 Curcumin promoted the expression of RXR-α and LXR-β in the hippocampus of APP/PS1 mice

Western blotting, qRT-PCR and immunocytochemistry were used to evaluate the expression of RXR-α and LXR-β in each group. The expression of RXR-α and LXR-β at the mRNA standard was significantly increased in Cur-L

and Cur-H groups compared with the control group (both $p < 0.05$) (Fig. 5A,B). Low-dose curcumin treatment increased the expression of RXR-α and LXR-β proteins in the hippocampus of APP/PS1 double transgenic mice (both $p < 0.05$). High-dose Curcumin increased the expression of RXR-α and LXR-β protein even more, as indicated by the increased intensity of immunocytochemical staining (Fig. 5C–I).

4. Discussion

In neuroscience, it is assumed that the critical pathological change in AD is the deposition of Aβ [15, 16]. Our earlier research showed that cognitive function was damaged and insoluble Aβ deposition gradually enhanced with age in the hippocampus of APP/PS1 mice [14]. Therefore, discovering a compound that reduces both Aβ deposition and cognitive impairment offers new hope for AD treatment. Curcumin is the main component of turmeric, a commonly used Chinese medicine derived from a plant compound. Many *in vitro* and *in vivo* experiments have shown that curcumin has anti-inflammatory, antioxidant, and anti-amyloid deposition properties [17]. Earlier studies found that curcumin reduces amyloid plaques by directly binding to and disaggregating amyloid and inhibiting the aggregation of Aβ [18]. Previous *in vitro* studies by our group found that curcumin can in-
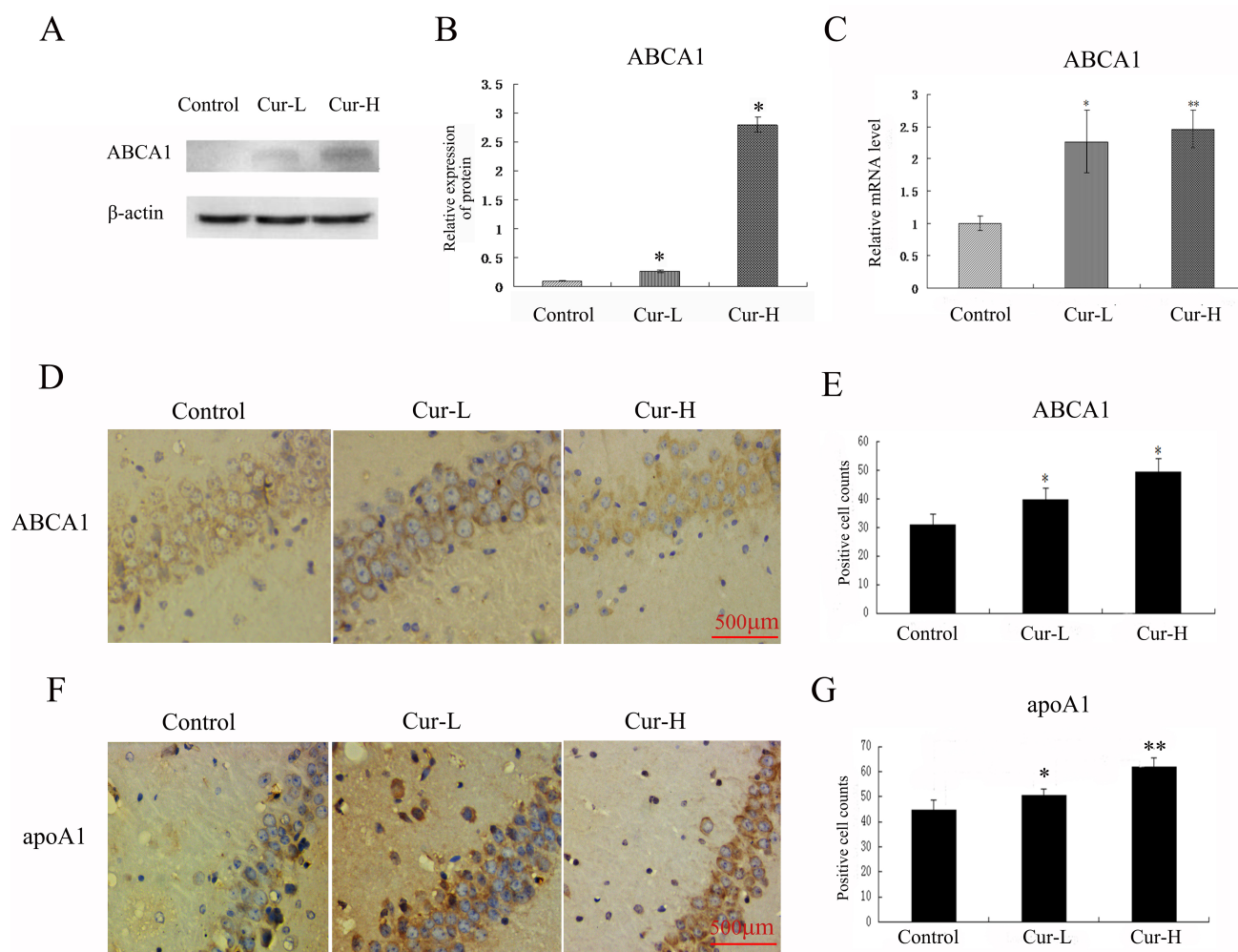
Fig. 4. Curcumin promoted the ABCA1 expression and apoA1 in the hippocampus of APP/PS1 mice. (A–C) Western blot and RT-PCR analysis to ABCA1 of each group. (D–G) Immunocytochemistry analysis of ABCA1 and apoA1 in each group (Scale bar = 500 μm). The data are mean ± SEM of a typical three experimental series (*$p < 0.05$, versus Control; **$p < 0.01$, versus Control).

hibit the production of Aβ via the PI3K/Akt/mTOR signaling pathway [19]. Therefore, many studies have shown that curcumin regulates the formation and aggregation of Aβ, but the exact mechanism remains unclear.

To further explore the protective effects of Curcumin against AD, the MWM test suggested that curcumin improved cognitive function in the hippocampus of APP/PS1 mice. Curcumin also reduced Aβ deposition in the hippocampus for mice. This result was consistent with our previous *in vitro* experiments [17].

Recent work suggests that AD is associated with cholesterol metabolism disorder. Cholesterol is an essential part of cell membranes and myelinated axons and plays an essential role in keeping the integrity and functionality of neurons [2]. However, the brain cannot obtain cholesterol from the blood due to the blood-brain barrier [4]. Synthesis by astrocytes and neurons is the primary source of cholesterol in brain tissues [20]. Epidemiological studies have found that patients with hypercholesterolemia were more likely to suf-

fer from AD and that taking cholesterol-lowering drugs could reduce the incidence of AD [21, 22]. Transgenic mice with hyper cholesterol and rabbits on a high-cholesterol diet both showed cholesterol metabolism disorder that could promote amyloid deposition and lead to AD and cognitive dysfunction [23, 24]. The total cholesterol (TC) and low-density lipoprotein (LDL) levels of AD patients were higher than those of ordinary people [25]. These studies suggested an association between AD and cholesterol metabolism disorder. In the present research, curcumin reduced the TC level in APP/PS1 mice serum, with high-dose curcumin being more effective than low-dose curcumin. This finding is similar to Shin *et al.* [26], who found that curcumin significantly lowered the cholesterol level.

The ABCA1 transmembrane system is a one-way cholesterol transporter that mediates outward transport after conjugating free cholesterol and phospholipid. ABCA1 is a member of the ATP-binding cassette family [27]. Structurally, it has two transmembrane segments and two nucleotide-
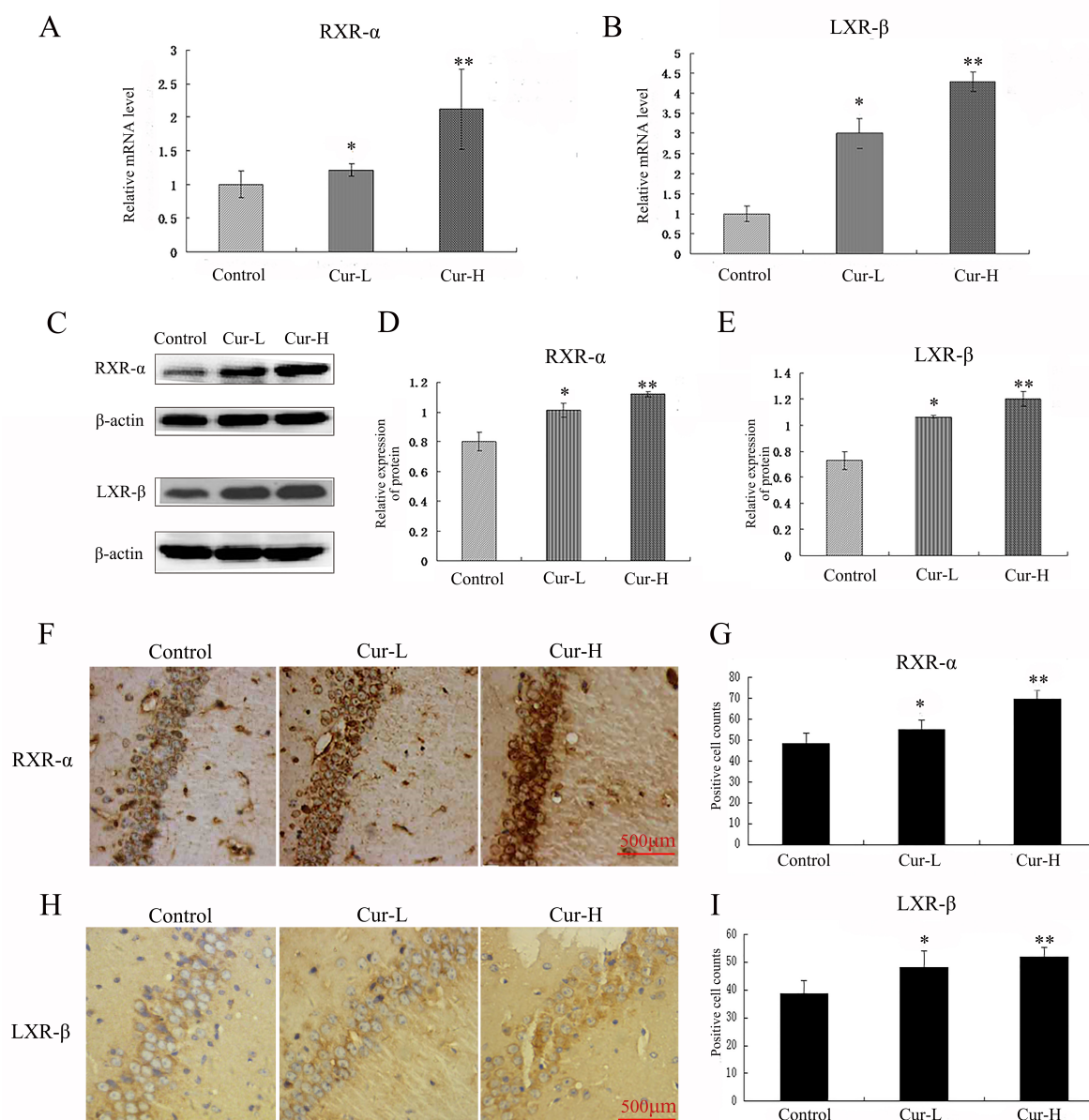
Fig. 5. Curcumin promoted the expression of RXR-α and LXR-β in the hippocampus of APP/PS1 mice. (A,B) Relative mRNA expression of RXR-α and LXR-β of every group. (C–E) Western blot analysis of RXR-α and LXR-β in each group. (F–I) Immunocytochemistry analysis of RXR-α and LXR-β in each group (Scale bar = 500 μm). The data stands for as mean $\pm$ SEM of a typical three experimental series (*$p < 0.05$, versus Control; **$p < 0.01$, versus Control).

binding domains. The nucleotide-binding site can bind ATP to provide sufficient energy to support the transmembrane transport of cholesterol. This way, ABCA1 substrates such as free cholesterol and phospholipid can be transferred to the cell exterior [28]. ABCA1 is mainly regulated by LXR and RXR. The former is a nuclear receptor for oxidized cholesterol, a member of the nuclear receptor superfamily, and a store of cholesterol content in many kinds of cells. LXR has two subtypes: LXR-α and LXR-β. LXR-β is widely distributed in the body and is highly expressed in the brain (2~5 times more than in the liver) [27, 29]. An increased cholesterol level in the brain can activate the LXR-β lig-

and, which in turn activates LXR-β. Activated LXR-β can form heterodimers with RXR, which then combine with specific DNA components (LXRE) to alter the expression of target genes such as ABCA1 [30]. RXR owns three subtypes, namely RXR-α, RXR-β, and RXR-γ. The former is mainly expressed in the hippocampus and neocortex, while the latter is only found in the neocortex [31]. Recent studies have shown that the LXR and ABCA1 cholesterol transport systems are essential in balancing nerve cells' interior and exterior cholesterol levels [32]. Moreover, the occurrence and development of AD were also closely related to this system.
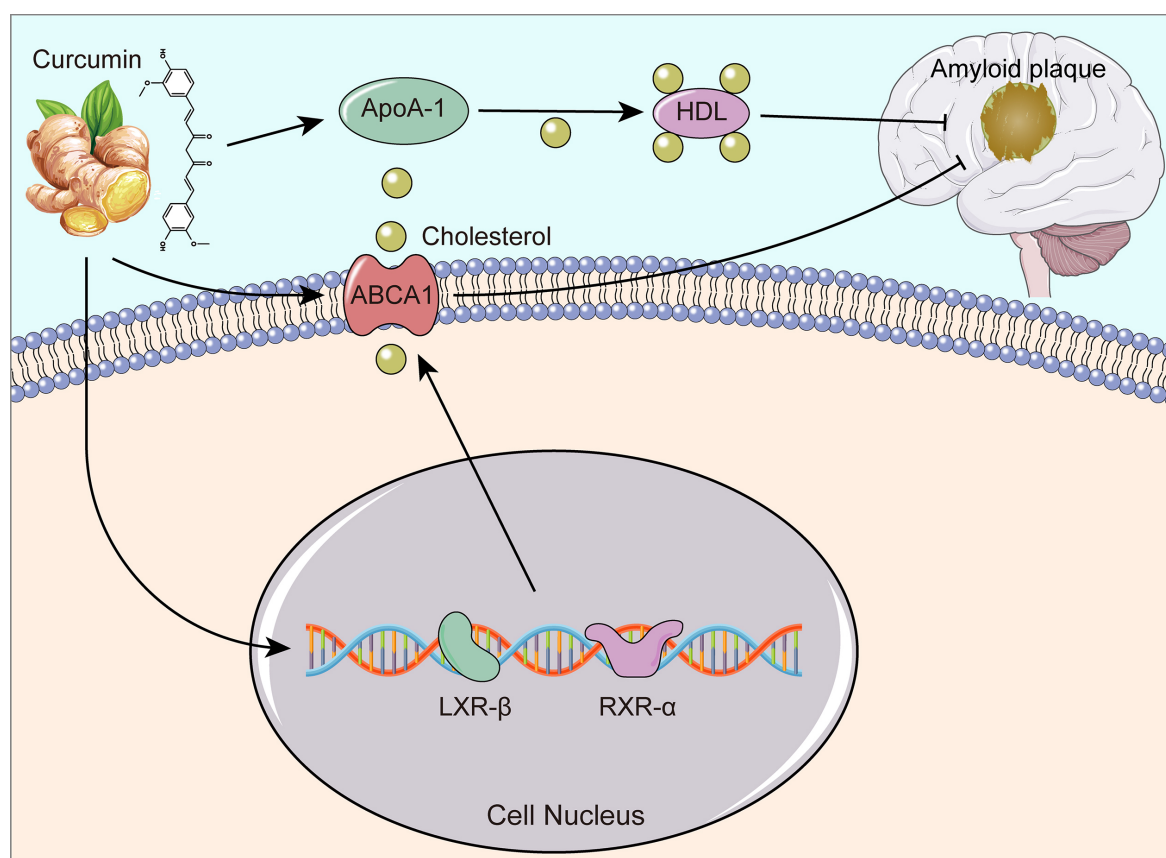
Fig. 6. Schematic diagram about the curcumin's possible mechanisms. Curcumin could promote the expression of LXR-β and RXR-α, which activated the ABCA1 cholesterol transmembrane system, thus maintaining the metabolic balance between intracellular and extracellular cholesterol. At the same time, curcumin reduces the serum total cholesterol level by increasing HDL levels and its source protein apoA1. In addition, the effect of curcumin on inhibiting the deposition of Aβ in the brain of mice may be achieved through the pathways mentioned above. Therefore, curcumin can improve lipid metabolic disorder and cognitive dysfunction by promoting the ABCA1 transmembrane transport system.

Jiang *et al.* [33] found that amyloid deposition in the brain of aging AD model mice (Tg2576) was dramatically decreased following treatment with GW3965, an agonist of LXR. At the same time, the memory capacity of these transgenic mice showed improvement. Fitz *et al.* [34] confirmed that treatment with LXR agonist could reduce amyloid deposition in the brains of transgenic mice fed a high-fat diet, as well as improve their cognitive function. Fitz *et al.* [34] also reported that LXR agonists could promote the activation of LXR-α/LXR-β *in vitro*. This activated the ABCA1 cholesterol transmembrane system, thus maintaining the metabolic balance between intracellular and extracellular cholesterol.

We observed increased expression of RXR-α and LXR-β at the protein and mRNA levels in the hippocampus of APP/PS1 mice fed curcumin for six months. Feeding with a higher dose of curcumin led to an even more significant increase. The production of Aβ decreased markedly after curcumin feeding, accompanied by increased LXR and ABCA1 expression. The ABCA1 cholesterol transmembrane transport system, therefore, plays a significant part in maintaining the balance of intracellular and extracellular cholesterol and participates in the transport and deposition of Aβ and the

formation of senile plaques. Hence, it could become a novel target for the treatment of AD. We also propose for the first time that curcumin is a natural agonist which increases the LXR-β and RXR-α expression. These can then be combined to promote the expression of ABCA1.

Following its transfer by ABCA1 to the extracellular fluid, cholesterol can combine with ApoA1 bound to the cell surface to form HDL. This carries cholesterol to the liver and reduces the cholesterol level due to the production of bile [34]. The generally accepted view is that increased HDL levels and its source protein apoA1 can lessen the probability of cardiovascular disease. Mature HDL is the smallest spherical particle amongst plasma lipoproteins and can scavenge intravascular lipid. Low HDL levels can lead to aberrant cholesterol metabolism and lipid deposition [35]. Recent clinical studies have shown that HDL and apoA1 levels in the plasma of AD patients are notably reduced [36]. Lewis *et al.* [37] previously reported the HDL level in APP/PS1/apoA1 transgenic mice was double that observed in APP/PS1 transgenic mice. These workers used the water maze test to confirm that APP/PS1/apoA1 transgenic mice did not appear to have age-related learning and memory deficits, in direct contrast to the

results for APP/PS1 transgenic mice. Lewis *et al.* [37] further suggested that plasma HDL and apoA1 levels were closely related to the cognitive function of AD patients. Our results also indicate that HDL has a protective effect against AD, since the HDL level increased as the level of Aβ decreased. Furthermore, the apoA1 expressed in the CA1 region to the hippocampus increased significantly in curcumin-fed mice, especially in the high-dose group. This observation is also consistent with the other findings mentioned above. The cholesterol carried by HDL and apoA1 is transferred from the cell interior by ABCA1.

5. Conclusions

Our results show that curcumin can improve lipid metabolic disorder and cognitive dysfunction by promoting the ABCA1 transmembrane transport system (Fig. 6). Furthermore, curcumin may become an ideal medication to prevent and treat AD due to its multitarget activity as a natural agonist in the ABCA1 transmembrane transport system. One outstanding question that warrants further study is whether the ABCA1 transmembrane transport system is the most important mechanism by which curcumin can ameliorate AD symptoms. Therefore, we will continue to explore the impacts of curcumin on the ABCA1 transmembrane transport system and lipid metabolism *in vivo*, and it offers new ideas for the treatment of AD.

Though there is essential evidence that curcumin may act on multiple pathways identified in AD's pathogenesis, its low bioavailability and low blood-brain barrier (BBB) penetration limit the role of Curcumin in AD [38], studies exploring nanoparticle technology found that liposomal curcumin, polymeric nano curcumin and poly-lactic-co-glycolic acid copolymer-curcumin could increase oral bioavailability or cross the BBB [39, 40]. Therefore, improving the bioavailability, BBB penetration and maintaining the half-life of curcumin is also a primary future goal.

Abbreviations

AD, Alzheimer's disease; ABCA1, ATP-binding cassette transporter A1; Aβ, β-amyloid; apoA1, apolipoprotein A1; APP/PSI, APPswe/PSEN1dE9; Cur-H, High-dose curcumin; Cur-L, Low-dose curcumin; HDL, High-density lipoprotein; LXR, Liver X receptor; LDL, Low lipoprotein; MWM, The Morris water maze; RXR, Retinoid X receptor; TC, Total cholesterol.

Author contributions

YL, FLZ, XZ and MYT designed the experiments; MYT, FLZ, ZPT, XZ, YW and CW performed the experiments; FLZ, MYT, ZPT and YYW helped in data analysis and paper writing. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Animals were raised and handled at the Chongqing University Cancer Hospital. All animal care protocols and exper-

iments were reviewed and approved by the Ethics Committee of Chongqing University Cancer Hospital (CZLS2021244-A). The consent for publication was obtained from all authors.

Conflict of interest

The authors declare no conflict of interest.

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