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Epigallocatechin-3-gallate Alleviates Cognitive Deficits in APP/PS1 Mice

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Summary: Alzheimer's disease (AD) shows cognitive impairments in clinic, which is multifactorial with different etiopathogenic mechanisms such as Aβ deposition, neuroinflammation and neuronal dystrophy involved. Therefore, multi-targets drugs with neuroprotective, anti-amyloidogenic and anti-inflammatory properties will be effective in AD treatment. Epigallocatechin-3-gallate (EGCG) possesses a broad spectrum of pharmacological activities in the prevention and treatment of multiple neurodegenerative diseases. In the present study, we showed that oral administration of EGCG (50 mg/kg) for 4 months significantly attenuated the cognitive deficits in APP/PS1 transgenic mice, which served as AD model. Moreover, EGCG induced an improvement in dendritic integrity and expression levels of synaptic proteins in the brain of APP/PS1 mice. And EGCG exerted obvious anti-inflammatory effects, which was manifested by alleviating microglia activation, decreasing pro-inflammatory cytokine (IL-1β) and increasing anti-inflammatory cytokines (IL-10, IL-13). Furthermore, β-amyloid (Aβ) plaques were markedly reduced in the hippocampus of 6-month old APP/PS1 mice after EGCG treatment. In conclusion, these findings indicate that EGCG improves AD-like cognitive impairments through neuroprotective, anti-amyloidogenic and anti-inflammatory effects, thus is a promising therapeutic candidate for AD.
Key words: Alzheimer's disease; cognitive impairments; epigallocatechin-3-gallate; anti-inflammation; β-amyloid

Alzheimer's disease (AD) is the most common neurodegenerative disease that causes progressive cognitive impairment and memory loss in the elderly. The etiopathogenic mechanisms of AD are complex, and two of the histopathological features in AD brains are extracellular senile plaques and intracellular neurofibrillary tangles (NFTs)[1–3]. The senile plaque is composed of β-amyloid (Aβ), which is formed by cleavage of the amyloid precursor protein (APP). Since its cleavage site is located within the Aβ sequence, α-secretase cleaves APP to generate a C-terminal fragment (C83) and an N-terminal fragment (sAPPα), thus, Aβ production is prevented. On the other hand, β-secretase (BACE1) cleaves APP to generate a C-terminal fragment (C99) and an N-terminal fragment (sAPPβ)[4, 5]. Subsequently, γ-secretase cleaves C99 to produce neurotoxic Aβ40/Aβ42, which further aggregate and lead to plaques generation[6]. NFTs consist of hyper-phosphorylated tau protein. Several post-mortem researches have demonstrated that the phosphorylation level of tau (per mole) increases to 5–9 phosphate groups in AD brains from those in normal, which is 2–3 phosphate groups[7–9]. Hyper-phosphorylated tau is incompetent to bind to tubulin, leading to microtubule depolymerization. Furthermore, hyper-phosphorylated tau propagates in a prion-like manner and induces neuronal and synaptic toxicity[10, 11].

Except the above two characteristic pathological changes, neuro-inflammation has been gradually recognized as a fatal pathological factor in AD development. The inflammation occurrence in AD is accompanied with glial activation, inflammatory cytokines and reactive oxygen species (ROS) release, which ultimately causes neuronal degeneration and damage[12–14]. Multiple histological studies have reported that Aβ1-42 promotes neuro-inflammation and oxidative stress, including activating microglia and producing pro-inflammatory cytokines and ROS. In addition, inflammatory cytokines also contribute

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to increased β-amyloid production and plaque formation[15–20]. Consequently, anti-inflammation has progressively sparked a great surge of interest in AD intervention.

Currently, there are no effective therapeutic agents for preventing or delaying the progression of AD, and a majority of the available therapeutic drugs are predominantly symptom-oriented, which do not address the underlying pathological changes. Besides, multiple of side effects which often increase in a dose-dependent manner limit the usage of the relevant drugs[21, 22].

Epigallocatechin-3-gallate (EGCG), the active polyphenol component of green tea, has addressed much attention due to its potential beneficial effectiveness, such as some positive roles in anti-oxidation, radical scavenging, metal chelating, anti-carcinogen, anti-apoptosis and anti-inflammation[23–25]. Numerous researches have brought the finding into light that EGCG slows down the process of brain aging[26, 27], and decreases Aβ level via upregulating the non-amyloidogenic α-secretase proteolytic pathway[28, 29]. Here, we report that oral administration of EGCG (50 mg/kg) for 4 months promotes synaptic function, and alleviates Aβ toxicity and inflammation, leading to an improvement of cognitive deficits in APP/PS1 mice. These data provide new clues to the mechanism of AD cognitive dysfunction, thereby providing an insight for therapeutic intervention.

1 MATERIALS AND METHODS

All antibodies used in the study are listed in table 1.

1.1 Animals and EGCG Administration

Two-month old C57 mice (*n*=9) and APP/PS1 mice (*n*=6), and EGCG administrated APP/PS1 mice (*n*=6) were kept at 23°C under a 12:12 h light/dark cycle. APP/PS1 mice were freely accessible to EGCG (Yuanye, China; 50 mg/kg daily) dissolved in H_2O or H_2O alone. Non-transgenic mice received H_2O alone. All mice were given behavioral tests and sacrificed at 6 months of age for biochemical and morphological analyses. Animals were housed and maintained in Medicine Animal Center of Jianghan University and all experiments were approved by the Medical Ethics Committee of Jianghan University.

1.2 Morris Water Maze Assay

Spatial learning and memory were tested using the Morris water maze (MWM) assay (XR-XM101, Xinruan, China) according to the classic Morris protocol[30]. For the spatial learning, mice were trained at a different starting position, spaced equally around the water pool, to find the submerged platform in 60 s. If the mice did not find the platform, they were guided to the platform and stayed on it for 30 s. The training procedure lasted for 5 days and 4 trials were performed each day. The time spent to locate the platform was recorded as latency to platform. After training, spatial memory retention was tested in the probe trial test. Mice were placed in the pool while the platform had been removed. The swimming path and the time spent in the target quadrant and the numbers of platform crossings were recorded.

1.3 Open Field and Novel Object Recognition (NOR)

Mice were placed into an open field box (XR-XZ301, Xinruan, China) and automated tracking was measured for 5 min. The diameter and height of the box were 50 cm. The total motion distance of mice and static time were recorded. Next day, two identical objects were introduced to the open field box. Mice were replaced into the chamber to explore objects for 5 min. On the following day, mice were put back to the chamber with one of the two objects supplanted by a

Table 1 All antibodies used in the study

Antibody	Specificity	Type	Dilution	Source
MAP2	Recognizing endogenous levels of MAP2 protein	mAb	1:200 for IF	CST (USA)
Snapsin-1	Detecting Synapsin-1 protein	mAb	1:1000 for WB	CST (USA)
Synaptophysin	Detecting Synaptophysin protein	mAb	1:1000 for WB	CST (USA)
Synaptotagmin	Detecting Synaptotagmin protein	mAb	1:1000 for WB	CST (USA)
PSD93	Recognizing endogenous levels of PSD93 protein	mAb	1:1000 for WB	CST (USA)
PSD95	Recognizing endogenous levels of PSD95 protein	mAb	1:1000 for WB	CST (USA)
GluR1	Recognizing endogenous levels of GluR1 protein	mAb	1:1000 for WB	CST (USA)
Iba1	Detecting Iba1 protein	mAb	1:200 for IF	Abcam (USA)
PS199	Phosphorylated Tau at Ser199	mAb	1:1000 for WB	Abcam (USA)
PS202	Phosphorylated Tau at Ser202	mAb	1:1000 for WB	Abcam (USA)
PS214	Phosphorylated Tau at Ser214	mAb	1:1000 for WB	Abcam (USA)
PS396	Phosphorylated Tau at Ser396	mAb	1:1000 for WB	CST (USA)
PS404	Phosphorylated Tau at Ser404	mAb	1:1000 for WB	CST (USA)
Tau5	Total-tau	mAb	1:1000 for WB	Immunoway (USA)
β-actin	Detecting β-actin protein	mAb	1:1000 for WB	Abcam (USA)
PT212	Phosphorylated Tau at Thr212	mAb	1:1000 for WB 1:200 for IHC	Thermo Fisher (USA)

mAb: monoclonal antibody; WB: Western blot; IF: immunofluorescence; IHC: immunohistochemistry

novel one. Mice behaviors were tracked for another 5 min. Discrimination index was calculated as follows: time exploring novel object minus time exploring familiar object divided by total time exploring novel and familiar objects.

1.4 Western Blotting

Hippocampi and cortexes were collected and stored at –80°C before process. Samples were homogenized in RIPA buffer [50 mmol/L Tris HCl (pH 8.0), 150 mmol/L NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS] containing protease (Beyotime, China) and phosphatase inhibitor (Beyotime, China). The protein concentrations of the homogenates were analyzed by the bicinchoninic acid (BCA) method. Same amount of protein was separated by SDS polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked with 3% non-fat milk for 1 h at room temperature, followed by overnight incubation with primary antibodies at 4°C and secondary antibodies for 1 h at room temperature. Immune bands were visualized using chemiluminescence reagents (Beyotime, China), followed by quantitative analysis by Image J2x.

1.5 Immunohistochemistry

Mice were transcardially perfused with 50 mL of physiological saline, followed by 50 mL of 4% paraformaldehyde (PFA). Brains were separated and post-fixed in PFA for 48 h and then sliced into 30-mm sections in phosphate buffered saline (PBS). Brain slices were subsequently washed by PBS and membrane-ruptured in 0.5% Triton-PBS, followed by being blocked using 5% bovine serum albumin (BSA) for 1 h at room temperature. Sections were then peroxidase treated, and immunostained with primary antibody (diluted in PBS) overnight at 4°C. Following three washing steps using PBS (10 min each), sections were then treated with appropriate mouse or rabbit HRP-conjugated secondary antibody and visualized with diaminobenzidine (DAB). Sections were dehydrated using graded ethanol, vitrificated by dimethylbenzene and mounted with coverslips.

For thioflavine-S histochemistry, sections were immersed in 0.25% $KMnO_4$ for 4 min. Following destaining in 1% $K_2S_2O_5$ and washing by 0.25% ethylic acid, sections of tissue were subsequently immersed in 0.0125% thioflavine-S (Yuanye, China; dissolved in 50% EtOH). Sections were then differentiated in 50% EtOH and cover-slipped using the fluorescent quenching medium agent (Beyotime, China). Sections were digitally scanned with Olympus microscope for analysis (Olympus, Japan).

1.6 Immunofluorescence

Brain slices were washed by PBS and subsequently blocked by 0.5% Triton-PBS containing 5% BSA for 1 h. Sections were then incubated overnight with primary antibody at 4°C, followed by incubation using the Alexa Fluor 488 (Beyotime, China) conjugated secondary antibodies for 2 h at room temperature. Following three washing steps using PBS, brain sections were redyed with Hoechst (Invitrogen, USA) for 5 min. Images were acquired using the Leica SP8 confocal microscope (Leica Microsystems CMS GmbH, Germany). The average values of fluorescence intensities from three images per sample were used for further quantification.

1.7 ELISA

Hippocampi and cortexes were homogenated in PBS (weight volume ratio=1 g:9 mL) and supernatants were collected after centrifugation for 20 min at 2000 r/min. Concentrations of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-4, IL-6, IL-10, and IL-13 were measured respectively, using ELISA assay kits for mice (Jiancheng Bioengineering Institute, Jiangsu), according to the manufacturer's protocols.

1.8 Statistical Analysis

Data were analyzed using SPSS 18.0 statistical software. All of the quantitative data were shown as mean±SEM. Statistical analysis was performed using either Student's *t* test (two groups) or one-way ANOVA procedure (more than two groups), followed by post hoc comparison. $P<0.05$ was considered as statistically significant.

2 RESULTS

2.1 EGCG Treatment Alleviated Cognitive Deficits in APP/PS1 Mice

To investigate the effect of EGCG on cognitive functions in APP/PS1 mice, we firstly evaluated the behavioral performances in APP/PS1 mice after oral administration of EGCG for 2 months. Open field was firstly employed to assess anxiety and activity levels, followed by NOR to measure short-term memory. In the open field test, there was no significant difference in total distance covered and static time among C57, APP/PS1 and EGCG treated APP/PS1 mice, suggesting that EGCG may not affect the level of anxiety in APP/PS1 mice (fig. 1A–1C). In the NOR test, EGCG-treated APP/PS1 mice showed a trend of increased exploration for the novel object without significant difference compared to that of APP/PS1 mice (fig. 1D). To test the effect of long-term EGCG treatment on anxiety, we carried out the open field test again after an additional 2 months of EGCG oral administration, and the results showed there were still no significant differences in anxiety and activity levels among the three groups (fig. 1E–1G). Next, we performed the MWM test to assess spatial learning and memory. The latency to the platform was reduced in EGCG-treated mice as compared with APP/PS1 mice (fig. 1H). In the probe test, we found a trend of increased target platform crossings (fig. 1I) and a remarkably

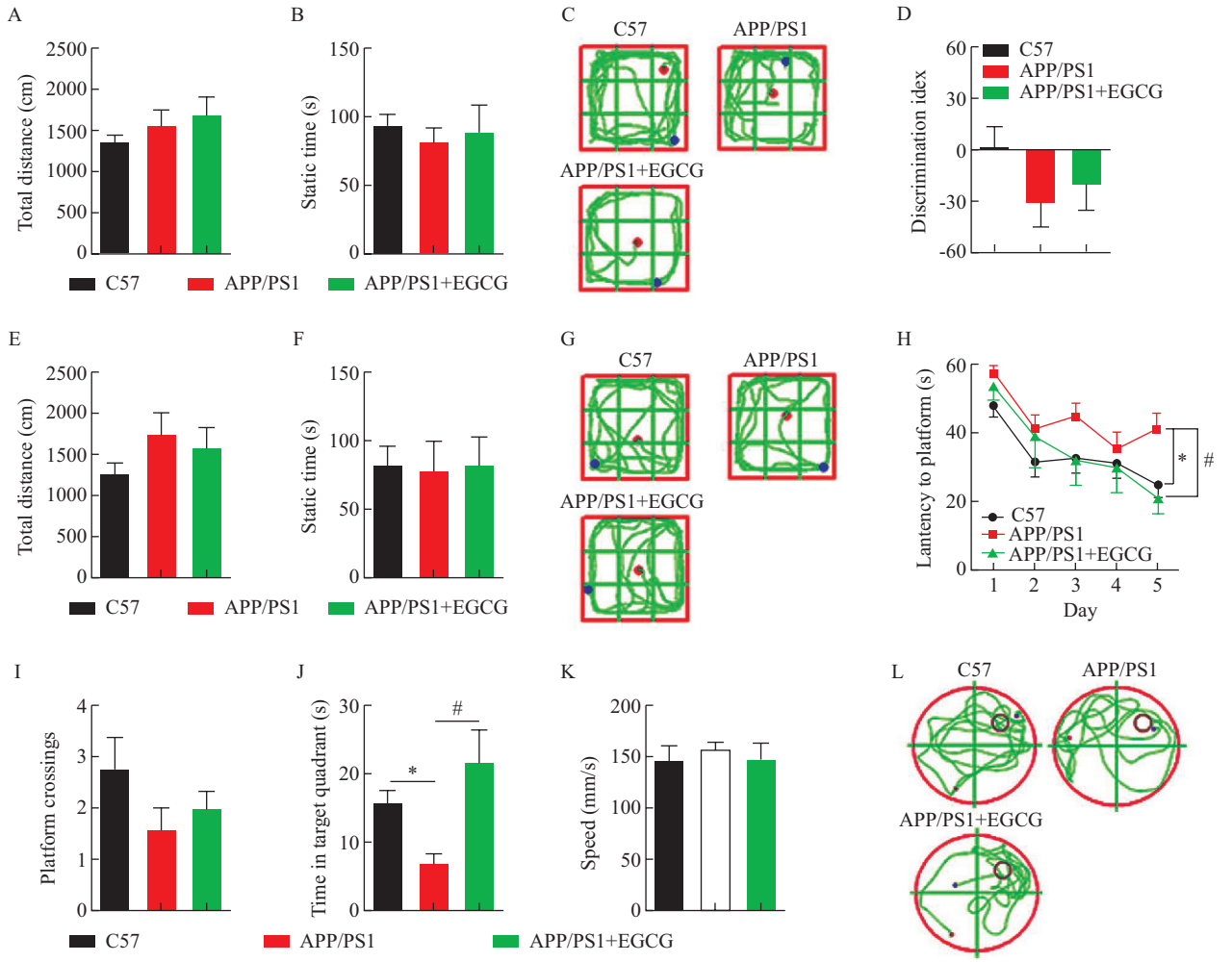

Fig. 1 EGCG alleviates cognitive deficits in APP/PS1 mice.

After oral administration of EGCG (50 mg/kg) in H_2O or H_2O alone for 2 months, open field (OF) test and novel object recognition (NOR) test were employed. Distance traveled (A) and static time (B) in the OF test were recorded. Representative traveled tracks were obtained from OF session (C). Discrimination index from C57, APP/PS1 and EGCG-administrated APP/PS1 mice was measred in the NOR test (D). After additional 2 months of EGCG oral administration, OF test and the Morris water maze (MWM) test were applied. Distance traveled (E), static time (F) and representative traveled tracks (G) in the OF test were recorded. For MWM assay, latency to find the platform (H) in the training, number of platform crossings (I), time spent in the target quadrant (J) and swimming velocity (K) in the probe test were measured. Representative swimming tracks were recorded in the probe test from MWM session (L). C57 mice (n=9), APP/PS1 mice (n=6), and EGCG-administrated APP/PS1 mice (n=6). $^{*}P<0.05$ *vs.* C57, $^{\#}P<0.05$ *vs.* APP/PS1

increased swimming time in the target quadrant (fig. 1J) in EGCG-treated group. However, there were no alterations of swimming speed among groups (fig. 1K). APP/PS1 mice of 6 months showed spatial learning and memory deficit compared to that of C57 mice (fig. 1H–1L). Together, EGCG administration might improve spatial learning and memory in APP/PS1 mice.

2.2 EGCG Attenuated Synaptic Impairment in APP/PS1 Mice

To investigate the potential mechanism underlying cognitive improvement by EGCG, synaptic integrality was detected by immunofluorescence staining with MAP2 (a dendritic marker) antibody. Immuno-staining results revealed a significant increase in dendritic density in CA1 of EGCG-treated mice compared to that in APP/PS1 group (fig. 2A and 2B). To further explore potential molecular mechanisms, several synapse-associated proteins in the hippocampus were measured. Western blotting showed a significant increase in synapsin-1, synaptophysin, PSD93 and GluR1 levels and an increasing tendency of synaptotagmin and PSD95 levels in the hippocampus of EGCG-treated APP/PS1 mice as compared with APP/PS1 mice (fig. 2C and 2D). These data imply that restoring of synaptic proteins may be associated with cognitive improvement in EGCG-treated APP/PS1 mice.

2.3 EGCG Reduced Inflammation Response in APP/PS1 Mice

Neuro-inflammation has been widely recognized as one of potential pathological factors in AD,

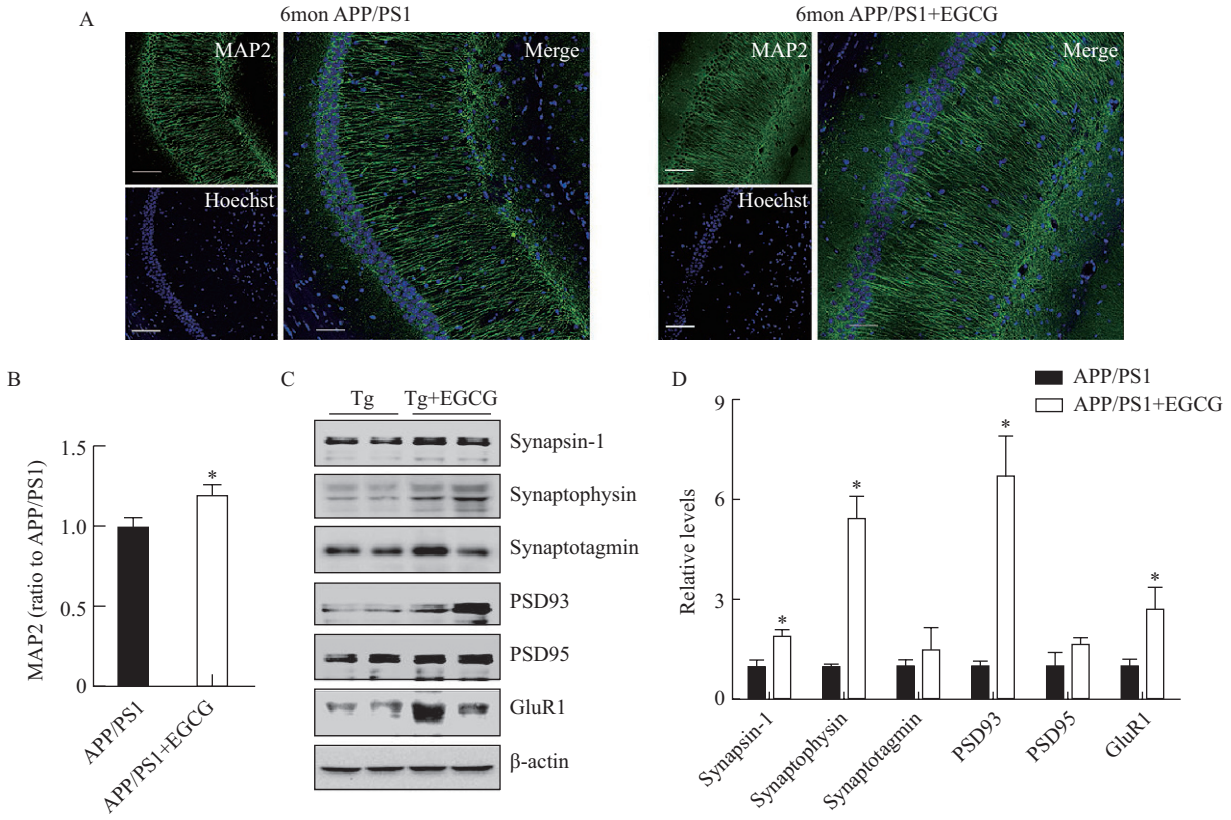

Fig. 2 EGCG attenuates synaptic impairment in APP/PS1 mice.
A: representative images of neurons stained with the dendritic marker MAP2 (green) and Hoechst labeling of cell nuclei (blue). Scale bar in left panel, 100 μm. Scale bar in right panel, 50 μm. B: bar graph of fluorescence intensity of MAP2 in the CA1 region of the hippocampus. *n*=3 mice/group. C: synaptic proteins levels in the hippocampus detected by Western blotting. D: quantification of synaptic proteins of panel C. *n*=3 mice/group. Bar graphs show mean±SEM. $^{*}P<0.05$ *vs*. APP/PS1

usually manifesting as microglia activation. To test whether EGCG affects the microglial function in the hippocampus of APP/PS1 mice, we analyzed Iba1 marker for microglia activation by immunofluorescence, and the results showed oral administration of EGCG significantly decreased the number of microglia cells in CA3 (fig 3A and 3C) and CA1 (fig. 3B and 3D) as compared with that in APP/PS1 mice without EGCG treatment. To further analyze anti-inflammatory effects of EGCG, the levels of pro-inflammatory (fig. 4A–4C) and anti-inflammatory (fig. 4D–4F) cytokines were measured by ELISA kits. It was found that IL-1β in the EGCG-treated mice was significantly reduced compared to that in APP/PS1 mice (fig. 4B), and anti-inflammatory cytokines IL-10 and IL-13 were significantly increased (fig. 4E and 4F). Together, these results suggest that EGCG treatment might improve the inflammation response in the brain of APP/PS1 mice.

2.4 EGCG Abated Amyloid Plaques in APP/PS1 Mice

Previous studies have shown that EGCG reduces β-amyloid generation *in vitro* and *in vivo*, through promoting the proteolytic activity of α-secretase and decreasing Aβ production. To determine whether oral administration of EGCG has similar effects against amyloid deposition in APP/PS1 mice, thioflavin-S staining was employed. Intriguingly, EGCG treatment significantly reduced Aβ plaques area in CA1 (fig. 5A and 5B) and CA3 (fig. 5C and 5D), as compared with that in APP/PS1 mice. These findings further supported that Aβ reduction is linked to cognitive improvement of APP/PS1 mice treated with EGCG.

2.5 EGCG Didn't Affect Tau Phosphorylation in APP/PS1 Mice

Abnormal phosphorylated tau is another pathological hallmark of AD, which is detected in the brain of 5–6-month-old APP/PS1 mice. To explore whether EGCG modulates tau phosphorylation in the hippocampus of 6-month-old APP/PS1 mice, we carried out immunohistochemistry and found that EGCG treatment did not alleviate tau phosphorylation in both CA1 and CA3 of the hippocampus compared to the non-treated group (fig. 6A and 6B). To verify the findings in immunostaining, we used homogenates of the hippocampi to analyze tau phosphorylation by Western blotting. As compared with the non-treated group, tau phosphorylation levels at Ser199, Ser202, Thr212, Ser214, Ser396 and Ser404, respectively, had

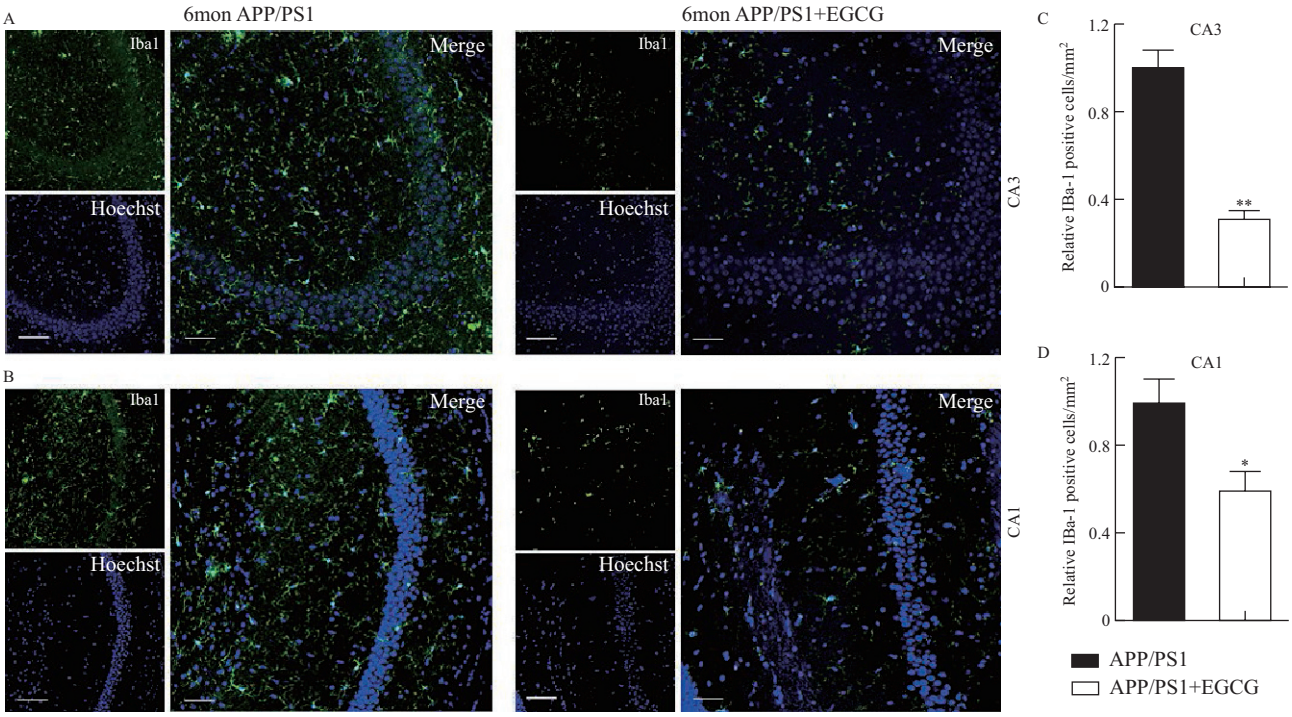

Fig. 3 EGCG reduces the number of microglia cells in hippocampus of APP/PS1 mice.
A and B: representative images of microglia stained with Iba1 (green) and Hoechst labeling of cell nuclei (blue) in the CA3 and CA1 regions. Scale bar in the left panel, 100 μm. Scale bar in the right panel, 50 μm. C and D: Statistic analysis of the Iba1-positive cell numbers. n=3 mice/group. Bar graphs show mean±SEM. $^{*}P$<0.05, $^{**}P$<0.01 *vs.* APP/PS1

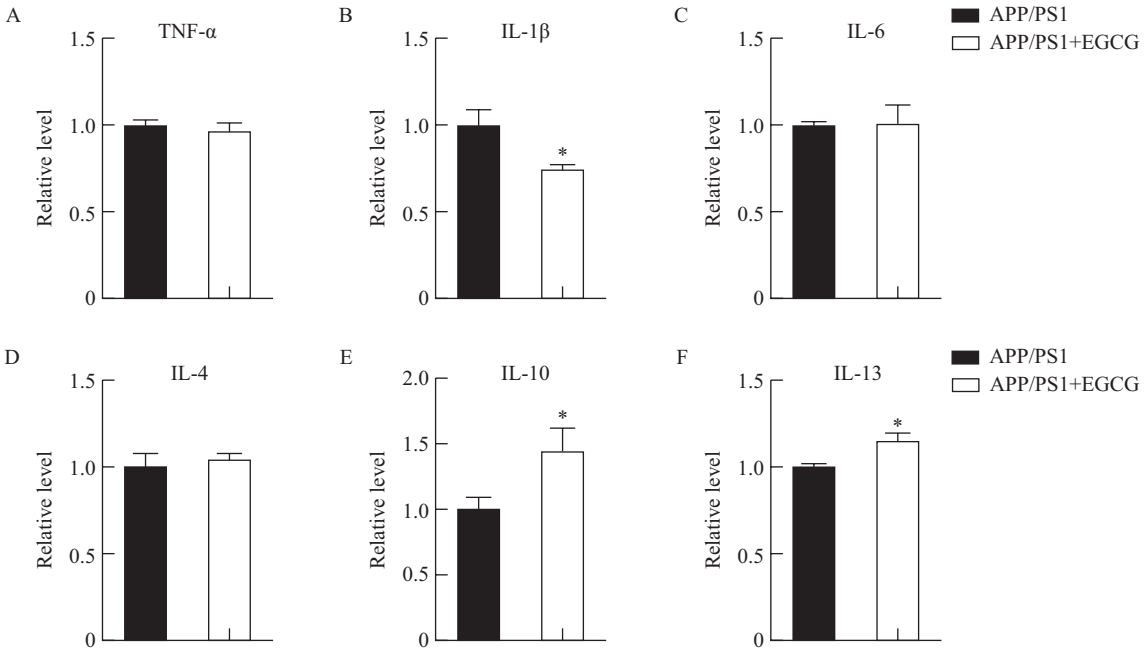

Fig. 4 EGCG modulates inflammatory cytokine.
A–C: levels of pro-inflammatory cytokines TNF-α (A), IL-1β (B) and IL-6 (C) in the mice brain. D–F: levels of anti-inflammatory cytokines IL-4 (D), IL-10 (E) and IL-13 (F) in the mice brain. n=3 mice/group. Bar graphs show mean±SEM. $^{*}P$<0.05 *vs.* APP/PS1

no significant changes in EGCG-treated mice (fig. 6C and 6D). These results indicate that long-term EGCG treatment does not affect tau phosphorylation in APP/PS1 mice at 6 months of age.

3 DISCUSSION

Increasing studies show that EGCG could serve as a potential therapeutic agent for preventing

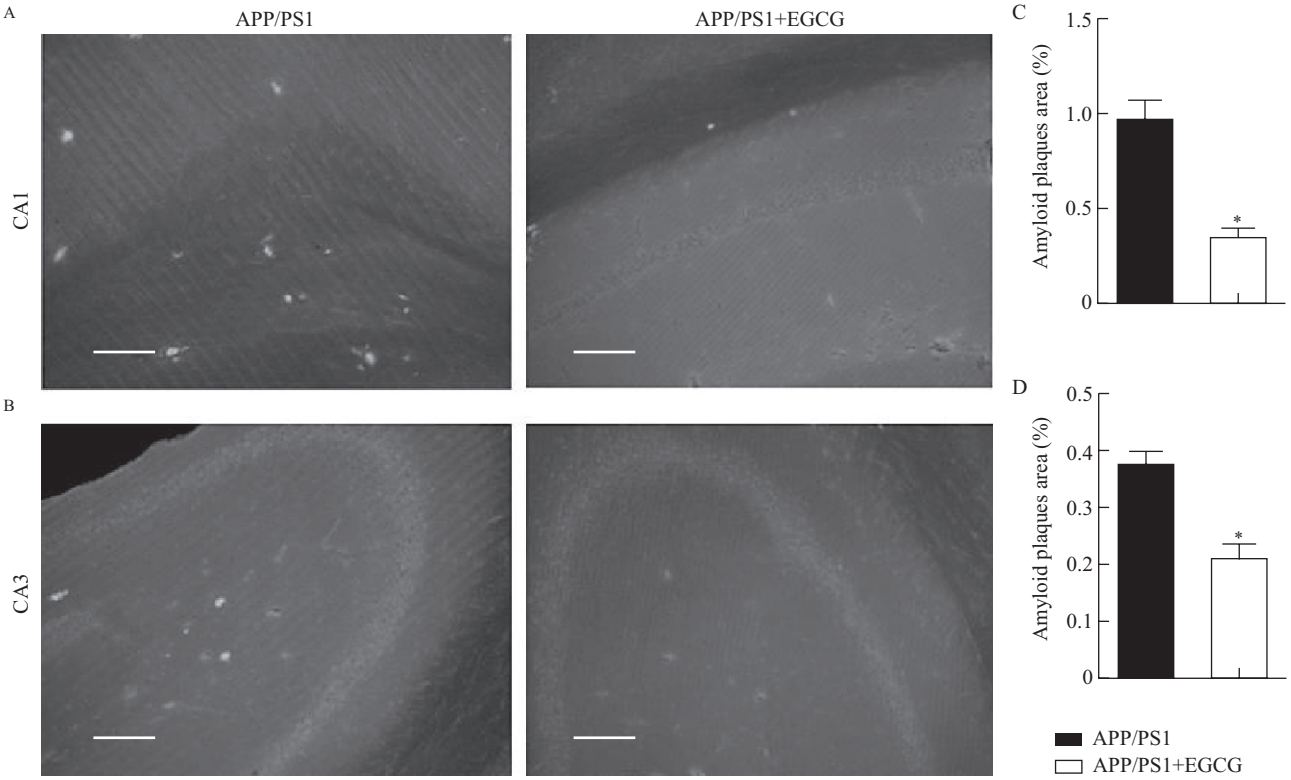

Fig. 5 EGCG reduces amyloid plaques in APP/PS1 mice.
Immunofluorescence of thioflavin S-stained Aβ plaques in the CA1 region (A) and the CA3 region (B) of the hippocampus (scale bar: 200 μm). Percentage of thioflavin-S plaques area (C and D) was quantified by image analysis. *n*=3 mice/group. Bar graphs show mean±SEM. $^{*}P<0.05$ *vs.* APP/PS1

neurodegeneration and cancer, mainly due to its beneficial effects on anti-oxidation, anti-carcinogen, anti-apoptosis and anti-inflammation[24–26]. In this study, we demonstrate that oral treatment of EGCG for 4 months (1) prevents cognitive deficit of APP/PS1 transgenic mice; (2) improves dendritic integrity and expression levels of synaptic proteins; (3) alleviates microglia activation and modulates the release of inflammatory cytokines; (4) reduces β-amyloid deposition.

Several human studies in Japan and the United States have illustrated that green tea consumption is inversely related to dementia and AD, which shows daily consumption of 2 or more cups of green tea reduced the prevalence of cognitive impairment[34–37]. EGCG, accounting for 50%–80% in a brewed cup of green tea, has been detected in brain tissue after oral administration of EGCG for a period of 5 days, suggesting that EGCG is capable to cross the blood-brain barrier[38, 39].

Aβ peptides and phosphorylated tau are the two basic pathological biomarkers of AD. Therefore, small molecular compounds that inhibit the formation or the neurotoxicity of these two biomarkers might be candidates to prevent or slow down the progression of AD. *In vitro* studies have demonstrated the protective effect of EGCG against Aβ-induced cytotoxicity via protein kinase B (PKB) activation or acetylcholine increase[40, 41]. EGCG has also shown the ability of preventing the conversion of Aβ1-42 monomers into a folded conformation, and consequently reducing Aβ fibrillation[42]. In addition, *in vivo* studies show that intraperitoneal injection or oral administration of EGCG provides a substantial reduction of Aβ levels in brain[28, 29]. Our present study also showed that oral administration of EGCG significantly decreased amyloid plaques in the hippocampus of APP/PS1 mice.

The study on rat primary cortical neurons showed that EGCG has the potentiality to clear phosphorylated tau through increasing related adaptor protein expression[42]. In Tg2576 transgenic mice, intraperitoneal injection with EGCG (20 mg/kg) also modulated soluble phosphorylated tau[28]. Our research did not reveal that orally administered EGCG (50 mg/kg daily) dissolved in H_2O for 4 months decreased tau phosphorylation in APP/PS1 transgenic mice. This might result from: (1) different routes of administration of EGCG; (2) different durations and concentrations of EGCG administration; (3) different strains of mice. Hence, it needs to be further investigated that which approach or concentration of EGCG is more efficacious to alleviate tau pathological process in AD.

Haque *et al* reported that 26 weeks of oral treatment

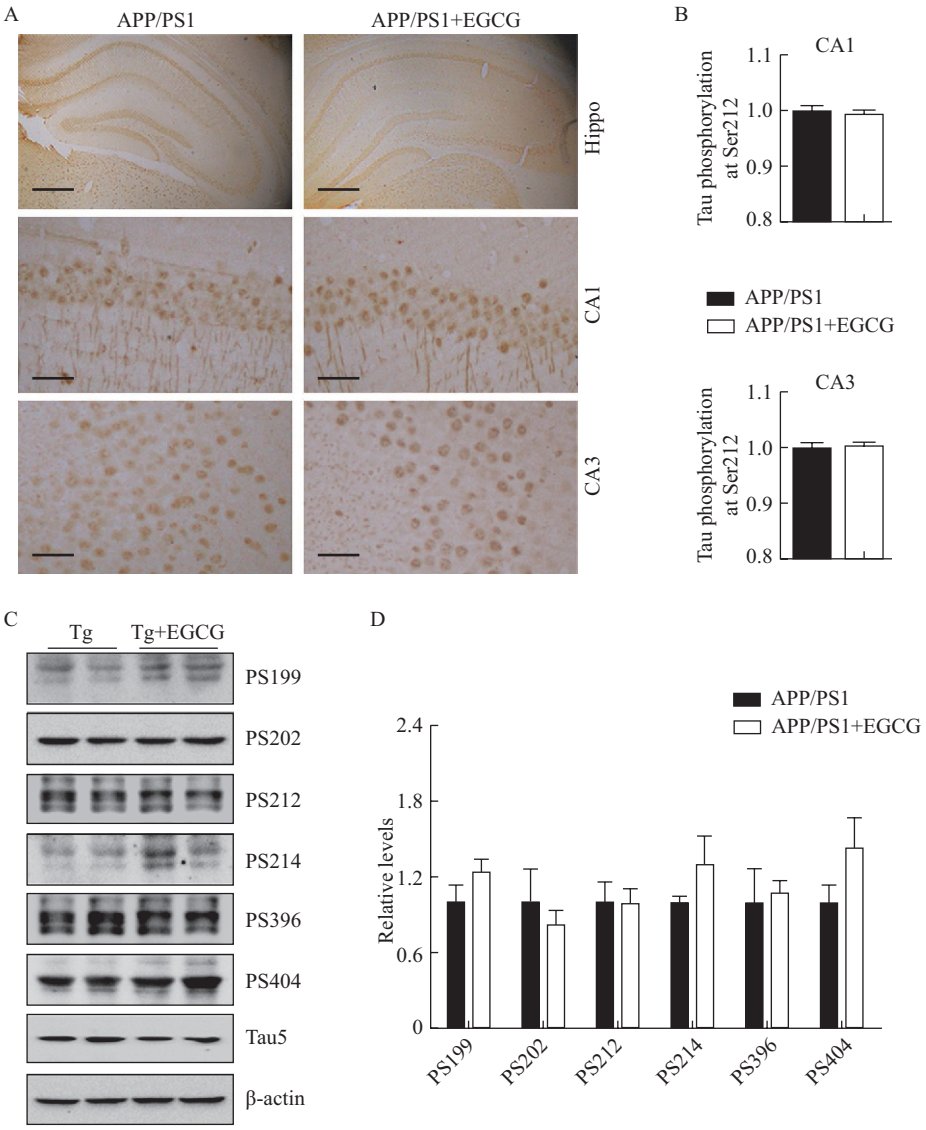

Fig. 6 EGCG does not modulate tau phosphorylation in APP/PS1 mice.
A: immunohistochemistry for the tau phosphorylation at Thr212 in the hippocampus (scale bar=200 μm), CA1 (scale bar=20 μm) and CA3 (scale bar=20 μm). B: bar graph of number of positive staining of PT212. *n*=3 mice/group. Data are presented as mean±SEM. C: representative blots of PS199, PS202, PT212, PS214, PS396, PS404, tau5 (total tau) and β-actin. D: quantification of relative tau phosphorylation levels. *n*=3 mice/group. Data are presented as mean±SEM.

with polyphenon E, of which the principal constituent is EGCG, significantly improved spatial learning ability in young rats[43]. In AD mice model, working memory performance was substantially improved through two months of intraperitoneal EGCG treatment from 12 to 14 months age or 6 months of oral treatment from 8 to 14 months age[28]. In the current study, we found that 4-month EGCG treatment alleviates spatial learning and memory deficits in APP/PS1 mice (from 2 to 6 months age). Our present study also showed long-term oral EGCG administration significantly improved dendritic density and several synapse-associated proteins in the hippocampus, which might account for cognitive improvement of APP/PS1 mice.

Long-term consumption of 2 or more cups of green tea daily can significantly increase antioxidant activity and decrease peroxides, lipid hydroperoxide and glutathione levels, suggesting that EGCG might upregulate the antioxidant levels and deter oxidative stress[44, 45]. EGCG can also inhibit leukocyte elastase through regulating the activation of matrix metalloproteinases (MMP), which triggers inflammation[46]. Oral administration of EGCG in murine model can also restrain neutrophil and inflammation in pulmonary fibrosis, and inhibit the pro-inflammatory enzyme myeloperoxidase in gut inflammation[47, 48]. These previous researches imply that EGCG could be a potential anti-inflammatory therapeutic agent. Besides Aβ deposits and tau phosphorylation, neuro-inflammation has been gradually recognized to play a

pivotal role in AD pathology[12–14]. Our current study showed 4-month administration of EGCG significantly decreased the count of microglia cells in CA3 and CA1 of APP/PS1 mice. As compared to those in non-treated APP/PS1 mice, the pro-inflammatory cytokine IL-1β was significantly reduced in the EGCG-treated mice, while the anti-inflammatory cytokines IL-10 and IL-13 were increased. Together, these results suggest that long-term oral administration of EGCG could attenuate the inflammation in the brain of APP/PS1 mice.

EGCG can protect cells against endoplasmic reticulum stress, mitochondrial dysfunction and glutamate-induced excite-toxicity. EGCG also exerts neuro-protective effects and preserves neuro-rescue activity[29, 49–51]. In addition, in contrast to most of the marketable therapeutic drugs with an extensive range of side effects, no available side effects of EGCG treatment have been reported yet. In conclusion, our study demonstrates that EGCG alleviates neuronal inflammation, amyloid plaques burden and neuronal degeneration in AD model mice. This study provides a basis of the molecular mechanism associated with cognitive impairments in AD and gives an insight for the treatment and prevention of AD.

Conflict of Interest Statement

The authors have no conflict of interest.

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