Quercitrin improved cognitive impairment through inhibiting inflammation induced by microglia in Alzheimer's disease mice

Lixin Wang[a,b,*], Jinxia Sun[a,b,*], Zhulei Miao[a,b], Xin Jiang[a,b], Yuejuan Zheng[b,c] and Guizhen Yang[a,b]

Objective **Diets rich in quercitrin show a neuroprotective effect, but the mechanism is not very clear at present. The objective of this study is to explore the effect and mechanism of quercitrin in the treatment of alzheimer's disease (AD).**

Methods **5XFAD transgenic mice were fed with a diet supplemented with quercitrin for three consecutive months. Behavioral experiments were conducted to assess the cognitive ability, luminex liquid chip technology was used to assess the production of proinflammatory cytokines and immunohistochemistry was used to elucidate the activation of microglia.**

Results **Quercitrin increased the frequency in exploring new objects, shortened the escape latency and increased the frequency crossing the platform in AD model mice. Quercitrin inhibited the activation and proliferation of microglia, inhibited the secretion of inflammatory cytokines and chemokines and reduced the accumulation of amyloid-β plaques in AD model mice.**

Conclusion **Quercitrin improved mice cognitive impairment through alleviating the intensity of inflammatory response and is a promising medicinal plant extract in the treatment of AD. *NeuroReport* 33: 327–335 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc.**

NeuroReport 2022, **33**:327–335

Keywords: Alzheimer's disease, inflammation, microglia, quercitrin

[a]Department of Immunology and Microbiology, School of Basic Medical Sciences, Shanghai University of Traditional Chinese Medicine, [b]Center for Traditional Chinese Medicine and Immunology Research, School of Basic Medical Sciences, Shanghai University of Traditional Chinese Medicine and [c]Shanghai Key Laboratory of Health Identification and Assessment, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Correspondence to Guizhen Yang, PhD, Department of Immunology and Microbiology, School of Basic Medical Sciences, Shanghai University of Traditional Chinese Medicine, 1200# Cailun Road, Pudong New District, Shanghai 201203, China
Tel: +86 021 51322150; e-mail: yanggzliuch@163.com

[*]Lixin Wang and Jinxia Sun contributed equally to the writing of this article.

Received 19 November 2021 **Accepted** 21 March 2022

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a marked deposition of amyloid-β (Aβ) plaques and progressive cognitive impairment. With the aging of society, the number of AD patients is increasing. By the middle of the 21st century, the number of people aged 65 and older with AD may grow to 13.8 million in USA, which brings a heavy economic and medical burden to the society [1]. Inflammatory responses are important mechanism in AD development. Both current and future potential treatments are based on the regulation of these pathways. Despite the significant public health issues that AD poses, only five medical treatments have been approved for this disease. Currently available drugs include glutamate and *N*-methyl-*D*-aspartic acid receptor antagonists, antioxidants and cholinesterase inhibitors, which act to control symptoms rather than to alter the course of AD [2]. There are many herbs with characteristics of multiple targets and fewer adverse effects than drugs currently available for AD [3]. Neuroinflammation is a secondary response caused by initial events of brain injury such as trauma, infection, and abnormal protein deposits such as Aβ plaques [4]. Emerging evidence suggests that inflammation is a driving force in the development of AD [5]. Activation and proliferation of glial cells stimulated by neuroinflammation is a central event in AD pathophysiology. Activation of microglia and astrocytes induces the secretion of proinflammatory factors such as IL-1β, TNF-α, granulocyte macrophage colony stimulating factor (GM-CSF), inducible nitric oxide synthase, nitric oxide, chemokine (c-c motif) ligand 5, CCL11 and chemokine (c-x-c motif) ligand 1, which conversely stimulates proliferation of glial cells [6]. The feedback loop between glial cells and inflammatory factors leads to the development of AD. In 1990s, epidemiological evidence suggested that anti-inflammatory drugs may have a protective effect in AD [7], and now the neuroinflammatory cascade is considered an important therapeutic target in the treatment of AD. Nonsteroid anti-inflammatory drugs (NSAIDs) are used in the treatment of AD due to inhibition of the inflammatory response in microglia and astrocytes and reduction of COX-2 expression in neurons [8]. Whether NSAIDs are effective against inflammatory factors remains to be determined. Based on

0959-4965 Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/WNR.0000000000001783

these premises, identification of novel drugs to prevent the onset or progression of neurodegenerative diseases is essential and urgent. Options are being searched in Chinese and Ayurvedic medicine [9]. Flavonoid is one of the largest families of natural products. The consumption of flavonoid-rich food throughout life may lower the risk of age-related neurodegenerative diseases [10]. Quercitrin is the most widely consumed flavonoid in the human diet. Quercitrin is primarily conjugated with carbohydrate and forms the backbone of other flavonoids such as rutin and tangeritin. High concentration of quercitrin is found in vegetables such as onion. Quercitrin is the most common form of extraction from plants. Multiple pharmaceutical effects of quercitrin, including antioxidant, anti-inflammatory and neuroprotection, have made it a promising food additive for the prevention of age-related disorders [11–13]. Researchers suggest that the memory of early-stage AD patients who consumed onion powder containing quercitrin was improved [14]. Quercitrin can potentially delay the onset of dementia and, therefore, markedly reduce its prevalence. However, whether quercitrin can treat AD is unclear. In this study, we investigated the effect and mechanism of quercitrin to protect AD.

Materials and methods

Chemicals

Quercitrin (high pressure liquid chromatography, purity > 98%) was purchased from Shanghai Tongtian Biotechnology Co., Ltd (Jiangsu, China), anti-Iba1 antibody was obtained from Wako (Osaka, Japan), biotin-rabbit antimouse IgG was purchased from Life Technologies (New York, USA), streptavidin conjugating horseradish peroxidase (SHP) was purchased from Invitrogen (New York, USA), protein precipitation solution and nuclei lysis solution were purchased from Promega (Wisconsin, USA), thioflavin-s and 3,3′-diaminobenzidine (DAB) were purchased from Sigma (Shanghai, China), agarose was purchased from Biowest (Loire Valley, France) and bio-plexpro mouse chemokine 23-plex panel was purchased from Bio-Rad (California, USA).

Animals and drug administration

Eight-week 5XFAD transgenic mice overexpressing human Aβ precursor protein with Swedish (K670N and M671L), Florida (I716V), London (V717I) familial Alzheimer's disease (FAD) mutations and human PS1 with M146L, L286V FAD mutations were purchased from Nanjing institute of biomedicine; wild-type (WT) C57BL/6J mice at the same age were the negative control mice. All mice were housed in the specific pathogen-free feeding room at Shanghai University of Traditional Chinese Medicine Animal Center. The diet supplemented with quercitrin was made by Jiangsu synergistic biology company. Mice were randomly divided into four groups, WT group (WT C57BL/6J mice fed with normal diet), AD group (5XFAD mice fed with normal diet) and quercitrin low/high dose (QL/QH) group (5XFAD mice fed with diet supplemented with 50 or 100 mg/kg/day quercitrin). Forty male mice were fed with diet with/without quercitrin for 3 months. Animal experiments were approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine, and experimental animal care followed their regulations.

Open field test

Mouse was placed in a 50 × 50 × 40 cm open-field box and let it move freely for 5 min. Movement trajectory was recorded and analyzed with the Ethovision XT 11.5 software. The experiment was repeated three times. The 20 × 20 cm area outside the central point of the box is the central area. Anxiety-like behavior was determined that mice spent more time outside the central area.

Novel object recognition test

The novel object recognition test was conducted with a previously described protocol and makes some changes [15]. Two same colored and shaped objects were placed in a 50 × 50 × 40 cm open-field box, and the mouse was allowed to explore objects freely for 5 min. After 1 and 24 h, one of the objects was replaced by a novel object with different colors but of similar size, and the mouse was placed back into the same box to move freely for 5 min. Mouse was exploring objects when the nose contacted with objects or the nose tip was less than 2 cm away from objects. Preference index was calculated as the ratio of time spending to explore one of objects over the total time spending to explore two objects (training phase) or the ratio of time spending to explore the novel object over the total time spending to explore novel and old objects (after 1 and 24 h of training phase).

Morris water maze test

The Morris water maze test was performed as previously described [16]. A 100 cm swimming pool was divided into four quadrants on average. The water temperature was maintained at 21 ± 1 °C. A 7-cm platform was located in the center of the VI quadrant and 1 cm above water at the first day, then was 1 cm under water from days 2 to 6. Mouse was placed into the pool from the I quadrant to find the platform within 60 s and stayed on the platform for 10 s. After a break, the mouse entered the pool from II and III quadrants, and the experiment was repeated. Positioning navigation experiment lasted for 5 days. Escape latency is the time spent to find the hidden platform after entering the pool. The escape latency and swimming trace were recorded by Ethovision XT 11.5 system (Noldus, Beijing, China). On the 6th day, the platform was removed, and the mouse was placed back into the pool from the center point of the II quadrant and swam freely for 60 s. The time spent in the VI quadrant, the frequency of crossing the platform was recorded.

Immunohistochemistry analysis

The mouse brain tissue was cut into 40 μm coronal sections. The sections were incubated in a mixture of PBS: formaldehyde: 3% H_2O_2 = 5: 4: 1 for 30 min, and

penetrated the membrane in 0.1% Triton X-100 solution for 10 min, blocked by 5% BSA at room temperature (RT) for 2 h. Sections were incubated overnight with Iba1 antibody at 4 °C. After rinsing with PBS, sections were treated with secondary antibody at RT for 2 h. After rinsing with PBS, sections were subsequently incubated with SHP at RT for 2 h and stained with DAB. Neutral resin sealed the slides after dehydration and degreasing. Images were captured with a LSM 880 microscope (Zeiss, Germany). The number of $Iba1^{+}$ microglia cells in the cortex and hippocampus was analyzed through the Image J software (Rawak Software Inc., Stuttgart, Germany).

Thioflavine-S fluorescent staining

Brain sections were stained with Thioflavine-S, as a previously described protocol [17]. Forty micrometer mouse coronal brain sections were attached to slides. The slides were immersed in 0.05% Thioflavine-S solution for 8 min, and immersed in 80% alcohol, washing the slides with deionized-diluted H_2O (ddH_2O). The brain sections were incubated in high phosphate saline buffer at 4 °C for 30 min. Fluorescent images were taken with a Zeiss microscope. The number and area of Aβ plaques in mouse brain were analyzed through Image J software.

Inflammatory factors detection

Brain tissue was placed in Eppendorf (Saxony, Germany) tu bes with 0.5 ml lysis solution containing RIPA and protease inhibitor. At least 90 μl of serum or brain tissue homogenate supernatant was prepared from each mouse. Inflammatory factor concentration in peripheral blood and brain was detected with bio-plexpro mouse chemokine 23-plex panel.

Statistical analysis

Statistical analysis was performed by the Graphpad software GraphPad Software Inc., San Diego, California, USA, results were given as means ± SD or standard error. Comparison between two groups was analyzed by Student's *t*-test. The difference between multiple groups was analyzed by one-way analysis of variance. Statistical significance was determined as $P < 0.05$ or $P < 0.01$.

Results

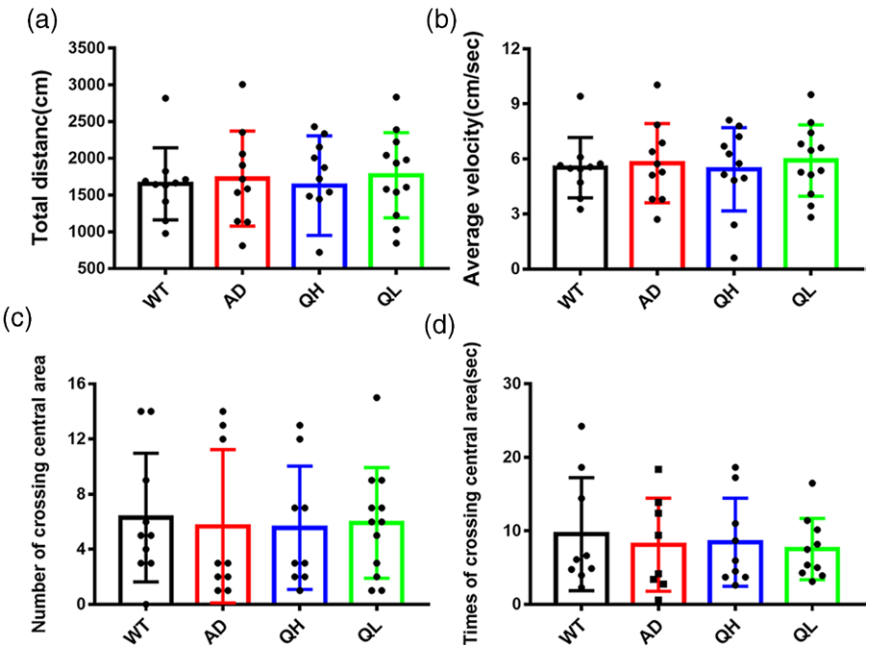
Quercitrin did not induce anxiety, depression in 5XFAD mice

We conducted an open field tests to evaluate the safety of quercitrin. As shown in Fig. 1, there was no significant difference in total movement distance ($F_{(3,39)} = 0.13$; $P = 0.94$; Fig. 1a), average velocity ($F_{(3,39)} = 0.13$; $P = 0.94$; Fig. 1b), number of crossings in the central area ($F_{(3,39)} = 0.15$; $P = 0.99$; Fig. 1c) and time spent in the central area ($F_{(3,39)} = 0.18$; $P = 0.91$; Fig. 1d) among the WT, AD, QL and QH groups. Therefore quercitrin is safe to treat mice because it does not induce anxiety and depression in 5XFAD mice

Quercitrin improved memory impairment in 5XFAD mice

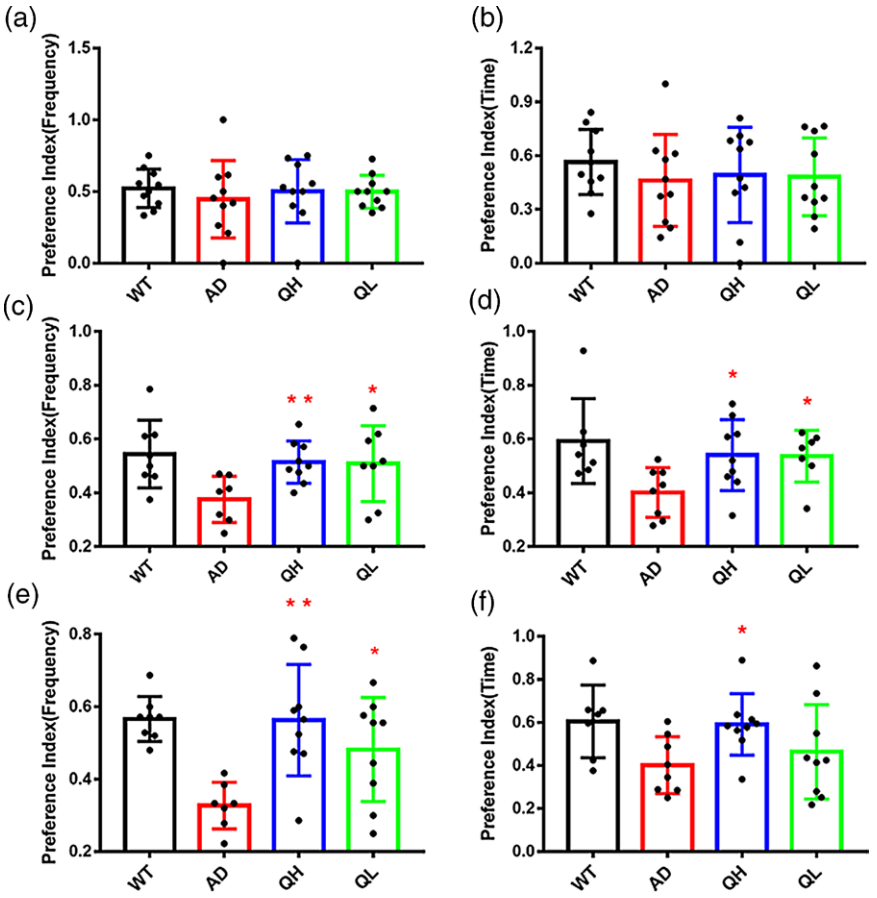
Short- and long-term memory impairment are the main clinical symptoms of AD patients. To evaluate the effect

Fig. 1



Quercitrin did not induce anxiety and depression in 5XFAD mice. (a) The total movement distance in the group of WT, AD, QH and QL. (b) Average movement speed. (c) Number of crossing central area. (d) Time of crossing the central area. Data are shown as the mean ± standard error ($n = 10$ in each group). AD, alzheimer's disease.

Fig. 2



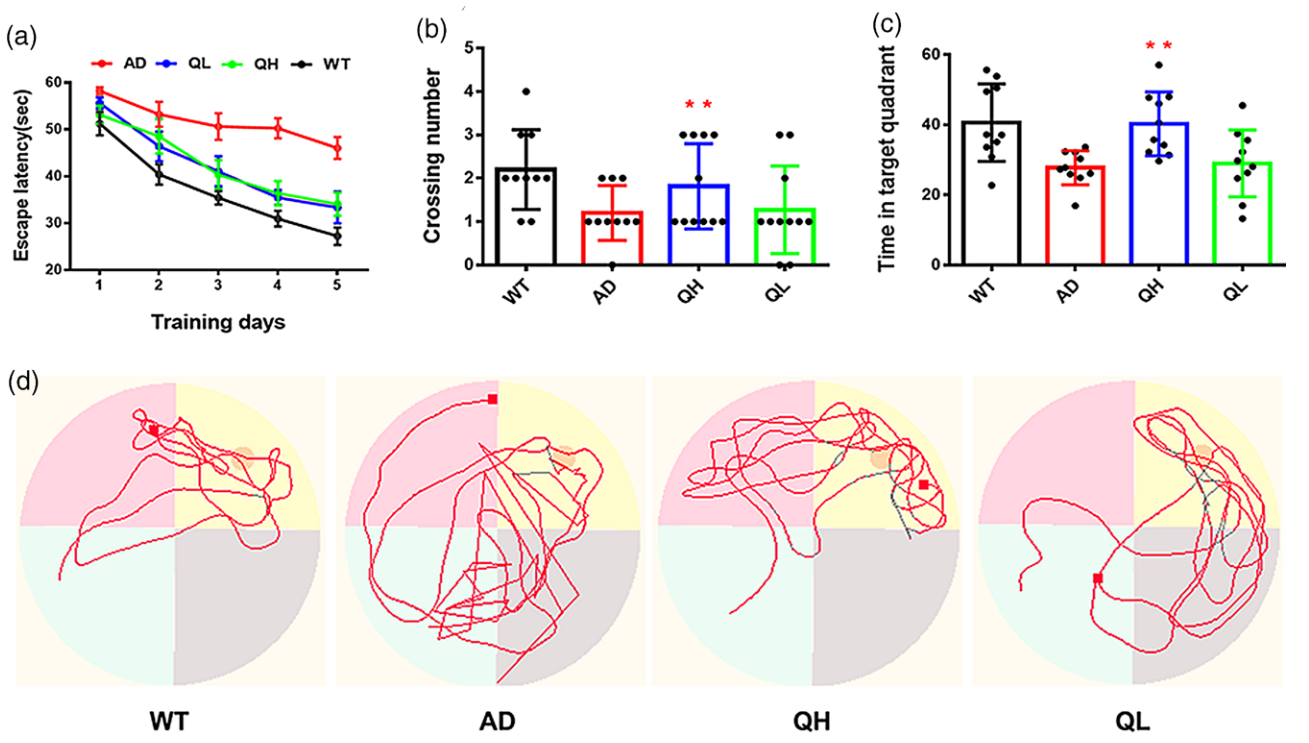
Quercitrin improved memory impairment in 5XFAD mice. (a) Percentage of frequency to recognize the new objects at the training phase. (b) Percentage of time to recognize the new objects at the training phase. (c) Percentage of frequency to recognize the new objects after 1 h of the training phase. (d) Percentage of time to recognize the new objects after 1 h of the training phase. (e) Percentage of frequency to recognize the new objects after 24 h of the training phase. (f) Percentage of time to recognize the new objects after 24 h of the training phase. Data are shown as the mean ± SD ($n = 10$ in each group). *$P < 0.05$ and **$P < 0.01$ vs. the AD group. AD, alzheimer's disease.

of quercitrin on memory improvement of AD mice, we carried out the novel object recognition test according to the characteristics that mice tend to explore the novel objects. We found that there was no difference in the nature of exploring the novel objects among the mice because there was no significant difference in the percentage of time ($F_{(3,39)} = 0.37$; $P = 0.77$) and frequency ($F_{(3,39)} = 0.27$; $P = 0.85$) exploring the novel objects among the four groups of mice at the training phase (0 h) (Fig. 2a and b). At the test phase (1 h after the training phase), the percentage of time ($F_{(3,39)} = 3.35$; $P = 0.033$) and frequency ($F_{(3,39)} = 4.35$; $P < 0.01$) exploring the novel objects decreased significantly in the AD group compared with that in the WT group, whereas increased in the QH group compared with that in the AD group. The results show that short-term memory of AD mice is impaired, quercitrin improves short-term memory of AD mice (Fig. 2c and d). At the test phase (24 h after the training phase), the percentage of time ($F_{(3,39)} = 3.04$; $P = 0.041$) and frequency ($F_{(3,39)} = 6.71$; $P < 0.01$) exploring the novel objects decreased significantly in the AD group versus the WT group, whereas increased in the QH group compared with that in the AD group. The results show that the long-term memory of AD mice is also impaired, and quercitrin improves long-term memory of AD mice (Fig. 2e and f).

Quercitrin improved spatial learning and memory impairment in 5XFAD mice

Spatial learning and memory impairment is also a common clinical manifestation of AD patients. We carried out Morris water maze test to evaluate the learning and memory improvement of quercitrin. In the stage of positioning navigation, the escape latency in the AD group was longer than the other three groups, but there was no significant difference between the four groups on the first day. In the next 4 days, the escape latency in the WT group was shorter and shorter, and the reduction range was the largest among the four groups. Although the escape latency in the AD group was also shortened, the reduction range was significantly less than that in the other three groups. The escape latency

Fig. 3



Quercitrin improved cognitive impairment in 5XFAD mice. (a) Escape latency in the water maze in the stage of positioning navigation. (b) Number of crossing the platform in the stage of spatial exploration. (c) Time spent in the target quadrant in the stage of the spatial exploration. (d) Representative real-time swimming trajectory in the stage of spatial exploration. Data are shown as the mean ± SD ($n = 10$ in each group). *$P < 0.05$ and **$P < 0.01$ vs. the AD group. AD, alzheimer's disease.

in the QH and QL group was shorter than that in the AD group, and the reduction range was larger than that in the AD group (Fig. 3a). In the stage of spatial exploration, the time spent in the target quadrant ($F_{(3,39)} = 6.14$; $P < 0.01$) and the frequency crossing the platform ($F_{(3,39)} = 4.91$; $P < 0.01$) decreased significantly in the AD group compared with that in the WT group, whereas increased significantly in the QH and QL group compared with that in the AD group (Fig. 3b and c). Representative real-time swimming trajectory of each group was shown in Fig. 3d. These results suggest that AD mice have surely spatial learning and memory impairment, quercitrin improves learning and memory impairment of AD mice.

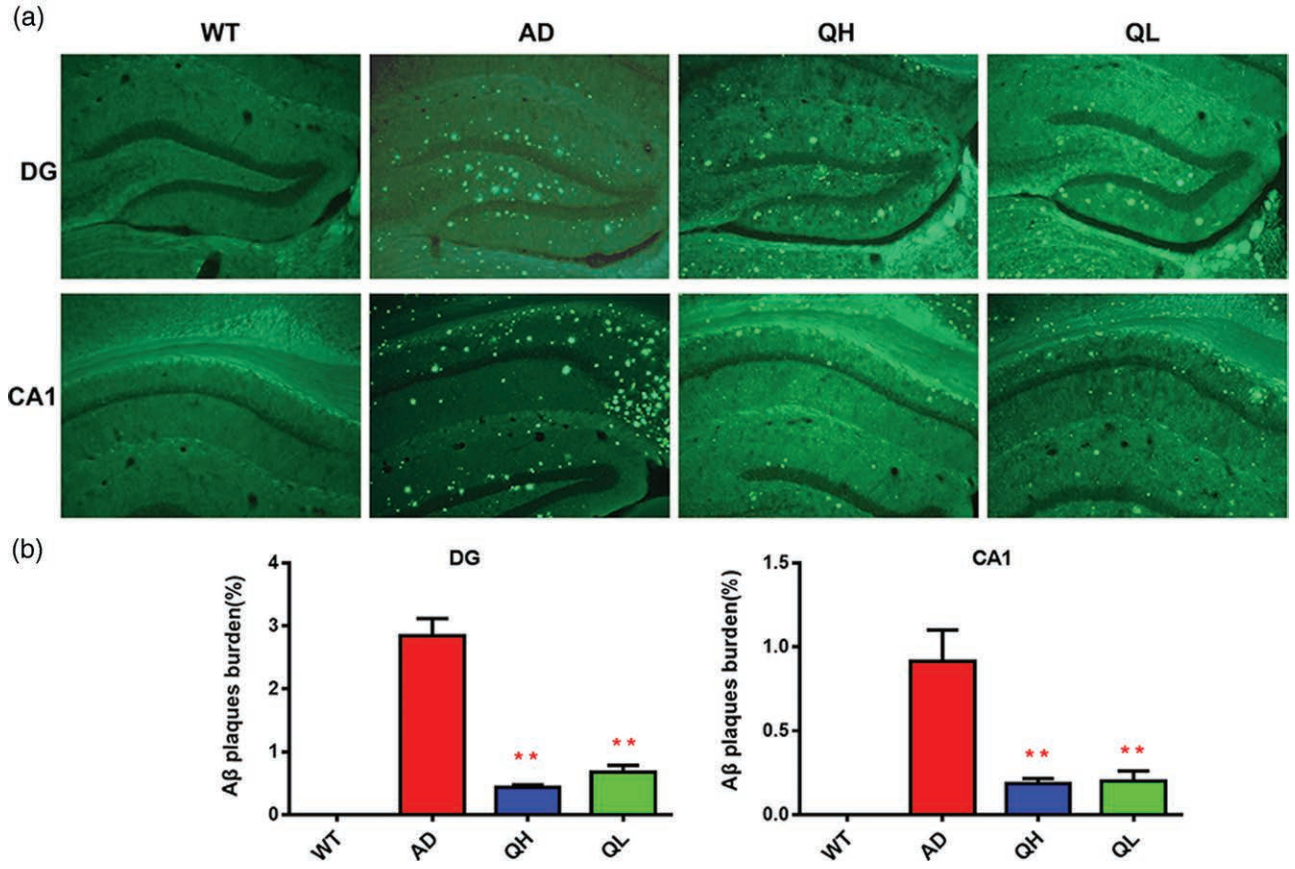
Quercitrin reduced the formation and accumulation of Aβ plaques in 5XFAD mice

The accumulation of Aβ plaques in the brain of AD patients is an important dangerous signal to stimulate the activation of microglia cells and secretion of inflammatory factors. Inhibition formation and accumulation of Aβ plaques would alleviate the intensity of neuroinflammation and delay the development of AD. To make clear the mechanisms of quercitrin to treat AD, the accumulation of Aβ plaques in the brain of AD mice after quercitrin treatment was analyzed. We found that the burden of Aβ plaques in hippocampus dentate gyrus (DG) ($F_{(3,39)} = 77.46$; $P < 0.01$) and CA1 ($F_{(3,39)} = 15.05$; $P < 0.01$) region increased significantly in the AD group compared with that in the WT group, however, decreased significantly in the QH and QL groups compared with that in the AD group (Fig. 4b). Representative immunofluorescent staining images of Aβ plaque accumulation in hippocampus DG and CA1 region in the group of WT, AD, QL and QH were shown in Fig. 4a. The results indicate that quercitrin reduces significantly the formation and accumulation of Aβ plaques in the brain of AD mice and can further relieve inflammation in the brain.

Quercitrin inhibited the activation of microglia in 5XFAD mice

Microglia is the main inflammatory cells in the brain, and activation and proliferation of microglia are the main cause of chronic, low-grade inflammation in AD patients. To confirm the anti-inflammatory effect of quercitrin, the number of $Iba1^+$ microglia in the anterior cingulate cortex (CAA), hippocampus DG and CA1 regions was analyzed. We found that the number of $Iba1^+$ microglia increased significantly, and more $Iba1^+$ microglia aggregated into clusters in the AD group compared with that in the WT group, whereas the number of $Iba1^+$ microglia was

Fig. 4



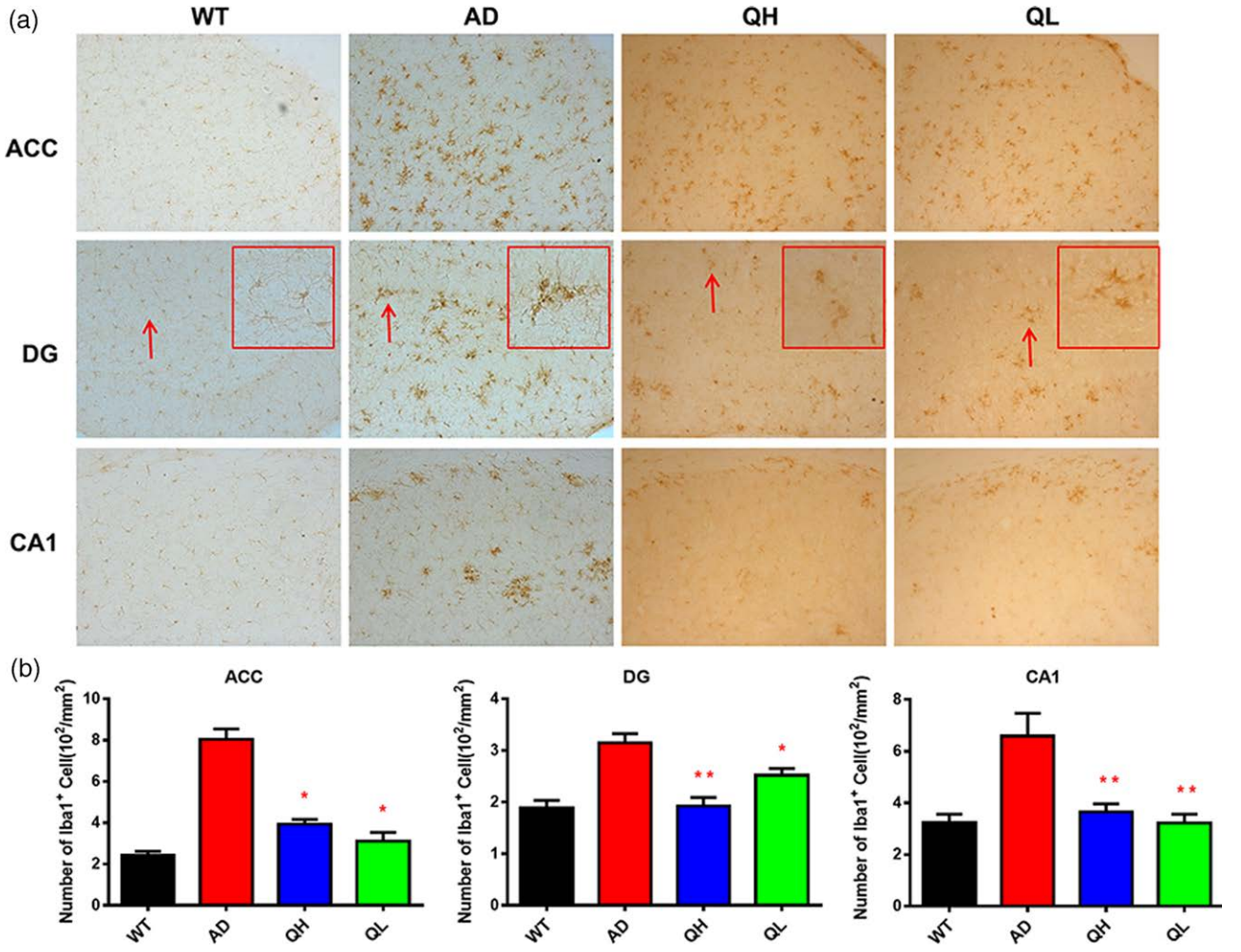
Quercitrin reduced the formation and accumulation of Aβ plaques in 5XFAD mice. (a) Representative immunofluorescent staining images of Aβ plaque accumulation in hippocampus DG and CA1 regions in the group of WT, AD, QH and QL. (b) Quantitative graph of Aβ plaque burden in hippocampus DG and CA1 regions in the group of WT, AD, QH and QL. Data are shown as the mean ± standard error ($n = 5$ in each group). *$P < 0.05$ and **$P < 0.01$ vs. AD group. Scale bar: 200 um. AD, alzheimer's disease.

decreased significantly in CAA ($F_{(3,39)} = 2.98$; $P = 0.045$), DG ($F_{(3,39)} = 14.26$; $P < 0.01$), CA1 ($F_{(3,39)} = 8.03$; $P < 0.01$) regions, and less Iba1$^+$ microglia aggregated into clusters in the QH and QL groups compared with that in the AD group (Fig. 5b). Representative immune-histochemical staining images of Iba1$^+$ microglia in the cortex, hippocampus DG and CA1 regions in the group of WT, AD, QL and QH were shown in Fig. 5a. The results indicate that microglia in the brain of AD mice are activated and proliferate massively, quercitrin can inhibit the activation and proliferation of microglia.

Quercitrin inhibited the secretion of inflammatory factors in 5XFAD mice

Over-activated microglia secrete inflammatory factors to promote neuron death. There are many proinflammatory cytokines, including IL-1β, IL-6, TNF-α, GM-CSF and MIP-1α, which play an essential role in recruiting glial cells to the sites of Aβ deposition, activating glial cells and inducing apoptosis of neurons. This study further analyzed the level of proinflammatory factors in the peripheral blood and brain of mice with the liquid suspension chip method. In the peripheral blood, the level of IL-1α ($F_{(3,20)} = 9.09$; $P < 0.01$), IL-6 ($F_{(3,20)} = 3.44$; $P = 0.036$), IL-10 ($F_{(3,20)} = 24.77$; $P < 0.01$), IL-17A ($F_{(3,20)} = 4.32$; $P = 0.017$) and G-CSF ($F_{(3,20)} = 22.83$; $P < 0.01$) increased significantly in the AD group than that in the WT group, whereas decreased significantly in the QH group compared with that in the AD group (Fig. 6a). The results show that the inflammatory reaction is obvious in the peripheral blood of AD mice, quercitrin can inhibit the inflammatory reaction in the peripheral blood of AD mice. In the brain, we found that the level of IL-1α ($F_{(3,20)} = 5.61$; $P < 0.01$), IL-4 ($F_{(3,20)} = 3.08$; $P = 0.041$), IL-6 ($F_{(3,20)} = 4.55$; $P = 0.017$), CXCL-1 ($F_{(3,20)} = 12.72$; $P < 0.01$), Eotaxin ($F_{(3,20)} = 4.96$; $P = 0.044$), G-CSF, MIP-1α ($F_{(3,20)} = 12.09$; $P < 0.01$) and MIP-1β ($F_{(3,20)} = 12.65$; $P < 0.01$) increased significantly in the AD group than that in the WT group, whereas the level of those inflammatory factors decreased in the QH group compared with that in the AD group (Fig. 6b). The results suggest that the inflammatory reaction is very serious in the brain of AD

Fig. 5



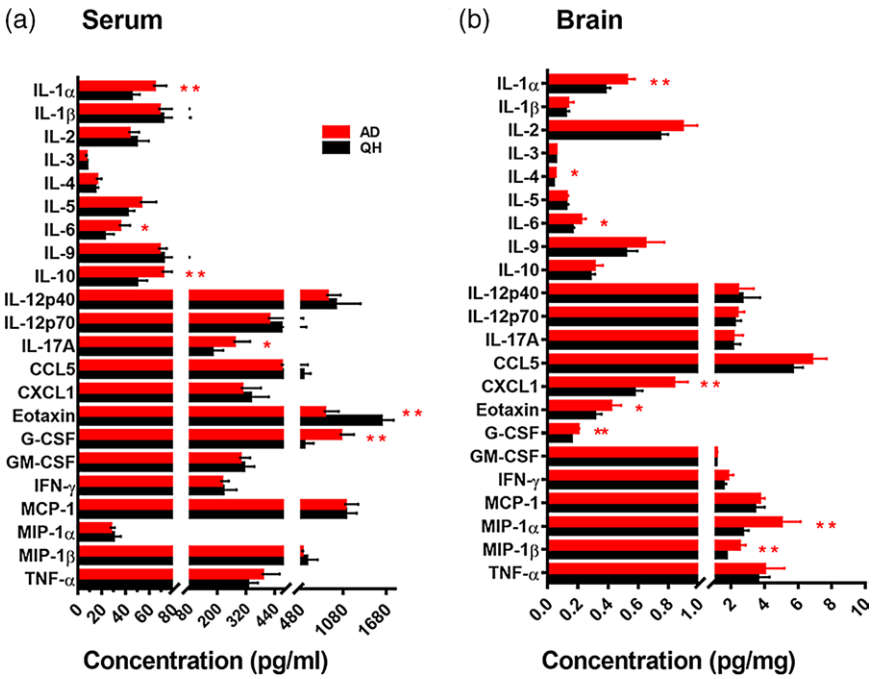
Quercitrin inhibited microglia activation in 5XFAD mice. (a) Representative immunohistochemical staining images of Iba1$^+$ microglia in the cortex, hippocampus DG and CA1 regions in the group of WT, AD, QH and QL. (b) Quantitative graph of the number of Iba1$^+$ microglia in the cortex, hippocampus DG and CA1 regions in group of WT, AD, QH and QL. Data are shown as the mean ± standard error ($n = 5$ in each group). *$P < 0.05$ and **$P < 0.01$ vs. AD group. Scale: 100 um. AD, alzheimer's disease.

mice, quercitrin can alleviate inflammation in the brain of AD mice. In a word, quercitrin alleviates the intensity of systemic inflammation in 5XFAD mice.

Discussion

Although our understanding about AD has increased dramatically over recent years, it remains far from fully understanding its pathogenesis. Scientists have been working for many years to develop drugs that target neurodegenerative diseases, however, there is a lack of effective drugs to treat AD. The study of natural compounds to prevent and treat AD has attracted extensive attention in recent years, and natural compounds are expected to bring new hope for drug development of AD treatment. Quercitrin distributes widely among plants and is common in daily diet. Modern Chinese medicine believes that quercitrin has the effects of relieving cough and asthma, detumescence and diuresis, clearing heat and detoxification, and increasing immunity. It can be used to treat chest tightness, shortness of breath, palpitation, palpitation and dreaminess. Several studies have reported the neuroprotective effect of quercitrin in ischemia, traumatic injury [18], Parkinson's disease [19] and Huntington's disease [20]. In the long-term medical practice, ancient Chinese doctors found that quercitrin also has the effect of significantly improving insomnia, amnesia and memory loss. Memory impairment is the main symptom of AD patients. Whether quercitrin can also improve memory loss in AD patients is still unknown. We found that 5XFAD mice fed with a diet added with quercitrin explored new objects more frequently than 5XFAD mice fed with a normal diet in

Fig. 6



Quercitrin inhibited the secretion of inflammatory factors in 5XFAD mice. (a) Quercitrin inhibited the secretion of inflammatory factors in the peripheral blood of 5XFAD mice. (b) Quercitrin inhibited the secretion of inflammatory factors in the brain of 5XFAD mice. Data are shown as the mean ± SD ($n = 6$ in each group), $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the AD group. AD, alzheimer's disease.

novel object recognition test, and 5XFAD mice fed with a diet added with quercitrin took less time to find platform than 5XFAD mice fed with a normal diet in the water maze test. These results indicate that quercitrin can improve the learning and memory impairment in AD mice. We further explored the mechanism of quercitrin protecting AD. Quercitrin has been shown to protect neurons from oxidative damage by regulating mitochondrial function [21,22] and inflammatory damage by inhibiting the secretion of inflammatory factors in model mouse of chronic unpredictable stress [23]. Chronic inflammatory reaction has been considered as one of the important pathogenesis of AD. Therefore, inhibition of inflammatory response has become one of the important ways to prevent and treat AD. In this study, we also found that quercitrin inhibited the level of IL-1α, IL-6, IL-17A and G-CSF in peripheral blood, and IL-1α, IL-4, IL-6, CXCL-1, Eotaxin, G-CSF, MIP-1α and MIP-1β in the brain, alleviated the intensity of systemic inflammation in 5XFAD mice. Furthermore, quercetin can more effectively inhibit inflammation in the brain because quercitrin permeates the blood–brain barrier effectively [24]. And in this study, 5XFAD mice were fed with a diet supplemented with quercitrin for three consecutive months, which will be beneficial for quercitrin to enter the central nervous system. Activated glial cells play a very essential role in the secretion of inflammatory factors in AD [25]. We confirmed that quercitrin inhibited the activation of microglia. In conclusion, quercitrin inhibits the activation of microglia, reduces the secretion of inflammatory factors, decreases the formation and accumulation of Aβ plaques, alleviates the systemic inflammation and improve cognitive impairment of AD mice. Because plant extracts have multidimensional anti-inflammatory effects and have few side effects after long-term use, quercitrin alone or combined with other drugs provide a new choice for the clinical treatment of AD. With the development of molecular biology, the research on the mechanisms of natural plant medicine has reached the cellular and molecular level, but the broad pharmacological activity of quercitrin makes it a big challenge to identify the targets in the cells, which leads to the lack of in-depth studies on the mechanisms of AD treatment by quercitrin. Just as there are many unknowns about AD, there are also many unknown fields about quercitrin in the treatment of AD, which will be the direction of our future efforts.

Conclusion

Quercitrin improved cognitive impairment in 5XFAD mice by inhibiting the activation of microglia, reducing the secretion of inflammatory factors, alleviating the systemic inflammation and reducing the formation and accumulation of Aβ plaques. Quercitrin could be a therapeutic candidate for AD.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China, Grant Number: 82073726.

Conflicts of interest

There are no conflicts of interest.

References

1 Vaz M, Silvestre S. Alzheimer's disease: recent treatment strategies. *Eur J Pharmacol* 2020; **887**:173554.
2 Stanzione P, Tropepi D. Drugs and clinical trials in neurodegenerative diseases. *Ann Ist Super Sanita* 2011; **47**:49–54.
3 Wang Q, Kuang H, Su Y, Sun Y, Feng J, Guo R, Chan K. Naturally derived anti-inflammatory compounds from Chinese medicinal plants. *J Ethnopharmacol* 2013; **146**:9–39.
4 Saito T, Saido TC. Neuroinflammation in mouse models of Alzheimer's disease. *Clin Exp Neuroimmunol* 2018; **9**:211–218.
5 Latta CH, Brothers HM, Wilcock DM. Neuroinflammation in Alzheimer's disease; a source of heterogeneity and target for personalized therapy. *Neuroscience* 2015; **302**:103–111.
6 Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; **8**:57–69.
7 McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 1996; **47**:425–432.
8 Trepanier CH, Milgram NW. Neuroinflammation in Alzheimer's disease: are NSAIDs and selective COX-2 inhibitors the next line of therapy? *J Alzheimers Dis* 2010; **21**:1089–1099.
9 Sharma R, Kuca K, Nepovimova E, Kabra A, Rao MM, Prajapati PK. Traditional ayurvedic and herbal remedies for Alzheimer's disease: from bench to bedside. *Expert Rev Neurother* 2019; **19**:359–374.
10 Rossi L, Mazzitelli S, Arciello M, Capo CR, Rotilio G. Benefits from dietary polyphenols for brain aging and Alzheimer's disease. *Neurochem Res* 2008; **33**:2390–2400.
11 Babaei F, Mirzababaei M, Nassiri-Asl M. Quercetin in food: possible mechanisms of its effect on memory. *J Food Sci* 2018; **83**:2280–2287.
12 Eid HM, Haddad PS. The antidiabetic potential of quercetin: underlying mechanisms. *Curr Med Chem* 2017; **24**:355–364.
13 Patel RV, Mistry BM, Shinde SK, Syed R, Singh V, Shin HS. Therapeutic potential of quercetin as a cardiovascular agent. *Eur J Med Chem* 2018; **155**:889–904.
14 Nakagawa T, Itoh M, Ohta K, Hayashi Y, Hayakawa M, Yamada Y, *et al.* Improvement of memory recall by quercetin in rodent contextual fear conditioning and human early-stage Alzheimer's disease patients. *Neuroreport* 2016; **27**:671–676.
15 Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, *et al.* Genetic enhancement of learning and memory in mice. *Nature* 1999; **401**:63–69.
16 Janus C. Search strategies used by APP transgenic mice during navigation in the Morris water maze. *Learn Mem* 2004; **11**:337–346.
17 Yang RY, Zhao G, Wang DM, Pang XC, Wang SB, Fang JS, *et al.* DL0410 can reverse cognitive impairment, synaptic loss and reduce plaque load in APP/PS1 transgenic mice. *Pharmacol Biochem Behav* 2015; **139**:15–26.
18 Li X, Wang H, Gao Y, Li L, Tang C, Wen G, *et al.* Protective effects of quercetin on mitochondrial biogenesis in experimental traumatic brain injury via the Nrf2 signaling pathway. *PLoS One* 2016; **11**:e0164237.
19 El-Horany HE, El-Latif RN, ElBatsh MM, Emam MN. Ameliorative effect of quercetin on neurochemical and behavioral deficits in rotenone rat model of Parkinson's disease: modulating autophagy (Quercetin on Experimental Parkinson's Disease). *J Biochem Mol Toxicol* 2016; **30**:360–369.
20 Sandhir R, Mehrotra A. Quercetin supplementation is effective in improving mitochondrial dysfunctions induced by 3-nitropropionic acid: implications in Huntington's disease. *Biochim Biophys Acta* 2013; **1832**:421–430.
21 Ansari MA, Abdul HM, Joshi G, Opii WO, Butterfield DA. Protective effect of quercetin in primary neurons against Abeta(1-42): relevance to Alzheimer's disease. *J Nutr Biochem* 2009; **20**:269–275.
22 Dong F, Wang S, Wang Y, Yang X, Jiang J, Wu D, *et al.* Quercetin ameliorates learning and memory via the Nrf2-ARE signaling pathway in d-galactose-induced neurotoxicity in mice. *Biochem Biophys Res Commun* 2017; **491**:636–641.
23 Rinwa P, Kumar A. Quercetin along with piperine prevents cognitive dysfunction, oxidative stress and neuro-inflammation associated with mouse model of chronic unpredictable stress. *Arch Pharm Res* 2017; **40**:1166–1175.
24 Kroemer HK, Klotz U. Glucuronidation of drugs. A re-evaluation of the pharmacological significance of the conjugates and modulating factors. *Clin Pharmacokinet* 1992; **23**:292–310.
25 Minter MR, Taylor JM, Crack PJ. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. *J Neurochem* 2016; **136**:457–474.