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Matrine improves cognitive impairment and modulates the balance of Th17/Treg cytokines in a rat model of Aβ_{1-42}-induced Alzheimer's disease

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Abstract

Matrine (MAT) has been reported for its anti-inflammatory and neuroprotective effects. However, little is known about its effects on Th17/Treg cytokines and cognitive impairment in Alzheimer's disease (AD). In the present study, we injected Aβ_{1-42} to the hippocampus of the rat to induce AD. Three groups of the AD rats were treated with MAT (25, 100 or 200 mg/kg/day, respectively) by intraperitoneal injection for 5 weeks. Levels of Th17 cell cytokines [interleukin (IL)-17A and IL-23] and regulatory T (Treg) cell cytokines [transforming growth factor β (TGF-β) and IL-35] in homogenates of the brain cortex and hippocampus were measured using enzyme-linked immunosorbent assay (ELISA) kits. The mRNA expressions of Th17 cell specific transcription factor RORγt and Treg cell specific transcription factor Foxp3 in the brain cortex and hippocampus were quantified by real-time RT-PCR. Learning and memory ability of the rats were evaluated by Morris water maze test and novel object recognition test. ELISA detections showed the AD rats had increased levels of IL-17A and IL-23 as well as decreased levels of TGF-β and IL-35. Matrine (100 and 200 mg/kg/day) significantly reversed the alternations of Th17/Treg cytokines induced by Aβ_{1-42} injection, decreased RORγt mRNA expression, increased Foxp3 mRNA expression and improved the learning and memory ability in the AD rats. The findings demonstrated that the AD rats had imbalance of Th17/Treg cytokines in the brain. MAT could dose-dependently restore the balance of Th17/Treg cytokines and attenuate the cognitive impairment in AD rats.

Key words: *matrine, Alzheimer's disease, cognitive impairment, Th17 cell, Treg cell, cytokine.*

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Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide [1]. China is facing the problem of an aging population, which leads to an increase in the number of people at risk of AD. Pathologically, deposition of extracellular amyloid-β (Aβ) in compact structure between neurons is one of the characteristics of AD [2]. Amyloid-β deposition in neocortex and hippocampus may cause neuronal death, ultimately leading to irreversible cognitive impairment and behavioral alterations.

Neuroinflammation is involved in the Aβ deposition mediated neuronal death. In AD brain, the insoluble Aβ peptide deposits provide obvious stimuli for inflammation [3]. Microglia is the innate immunocyte residing in the brain. It has been proved to play an important role in the inflammatory response of AD by secreting inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α) [4]. Besides microglia, T lymphocytes are also involved in the inflammatory response in the AD brain. Generally, peripheral T cells cannot migrate into the brain parenchyma due to the existence of blood-brain barrier (BBB). But it has been shown that activated T cells could easily cross the BBB and it was reported that T cell number in brain parenchyma of AD cases increased if compared to the cases with non-AD degenerative dementias [5, 6]. The role of Th1 cells in AD has been well studied. Recently, the importance of T helper (Th) 17 cells in the pathogenesis of AD is reported in several studies. Th17 cell is a Th cell subset which secretes cytokines IL-17, IL-21, IL-22 and IL-23 [7]. It has been demonstrated to play a pathogenic role in immune-mediated diseases [8]. Increased levels of transcription factor RORγt and cytokines IL-17 and IL-22 have been found in the brain of AD cases and the Th17 cell-mediated neuroinflammation is thought to be involved in neurodegeneration of Aβ_{1-42}-in-

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duced AD in an animal model [9, 10]. In addition, the role of regulatory T cell (Treg) which secrets anti-inflammatory cytokines IL-35, IL-10 and transforming growth factor β (TGF-β) in AD also has been addressed in literature. Generally, it is regarded that the Treg cell plays a protective role in AD [11]. A recent study by Yang showed that systemic transplantation of autologous Tregs was effective in AD. Very recent studies also showed TGF-β1 can protect against the neuroinflammation and the neurodegeneration in $A\beta_{1-42}$-induced AD animal models [12, 13]. In addition, the imbalance between Th17 cell and Treg cell has been well demonstrated to contribute to the pathogenesis of many diseases [14]. Yet, to our knowledge, little is known about the imbalances of Th17 cell and Treg cell in AD.

Due to the contribution of neuroinflammation to AD, anti-neuroinflammation is regarded as a potential strategy in treating AD. Many Chinese traditional herbs and extracts have anti-inflammatory activity and have been widely used in inflammatory diseases. Matrine (MAT) is a quinolizidine alkaloid derived from the herb Radix Sophorae Flave. Studies have shown that MAT has several pharmacological effects. It has been recently used to manage inflammation-mediated disorders in animals [15]. A study by Kan [16] reported that MAT protected neuro-axon from central nervous system (CNS) inflammation-induced injury. Moreover, Ni [17] found that MAT could protect the ultra-structure of hippocampal neuron in the AD rat. However, little is known about its effects on Th17/Treg cytokines and the cognitive impairment in AD subjects.

In the present study, we detected the protective effects of MAT on the impairment of learning and memory in AD animals and on the expression of Th17/Treg cytokines.

Material and methods

Animals

Sprague-Dawley rats obtained from the Anhui Medical University Laboratory Animal Center were used in this study. The animals were kept on a 12-hour light/dark cycle, housed in plastic cages (3 rats in a cage) under the set temperature (22 ±3°C) and humidity (55 ±5%) with free access to food and water. The experimental procedures were in accordance with the international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of Changyi People's Hospital.

Induction of $A\beta_{1-42}$-induced Alzheimer's disease rat model and matrine treatment

Animals were divided into a control group, sham-operated group (sham group), AD group, MAT 25 mg/kg group (MAT-25 group), MAT 100 mg/kg group (MAT-100 group) and MAT 150 mg/kg group (MAT-150 group) with 9 rats in each group. $A\beta_{1-42}$ (Sigma-Aldrich, USA) was prepared and injected as described in literature [10]. After anesthetization with chloral hydrate on day 1, the animal was mounted onto a stereotactic frame. One µl of $A\beta_{1-42}$ (dissolved in sterile saline) solution containing 4 µg $A\beta_{1-42}$ was injected (with an injection speed of 0.2 µl/min) to each side of the hippocampus of the rats in the AD group, MAT-25 group, MAT-100 group and MAT-150 group. Rats in the sham group received equal volume of the saline injection. Rats in the control group did not receive any injection. The stereotaxic coordinates for the injection are as follows: 3.6 mm posterior to the bregma, 2.4 mm left/right to the midline, and 2.8 mm ventral to the bregma. In order to avoid infection, the rat received an intramuscular injection of penicillin following the $A\beta_{1-42}$ administration. From day 1, MAT (Nanjing TCM Institute of Chinese Materia Medica, Nanjing, China) (dissolved in saline, freshly prepared before use) was daily administered to the animals at 3 different dosages (25 mg/kg for the MAT-25 group, 100 mg/kg for the MAT-100 group and 150 mg/kg for the MAT-150 group) by intraperitoneal injection for 5 consecutive weeks. Equal volumes of saline were intraperitoneally injected to the animals in other groups.

Morris water maze test

The water maze apparatus (180 cm in diameter, 45 cm high) was filled to a depth of 35 cm with 22 ±1°C water. The pool was divided into four equal quadrants with an invisible platform (10 cm in diameter, 2 cm below the water surface) in the northern quadrant. Spatial learning was tested from day 30 to 34 with 4 trials a day. The mice were forced to swim to find the hidden platform starting from the 4 different quadrants during the test. The rats were artificially guided to the hidden platform if they could not find it in 120 s. They were allowed to spend 30 s on the platform and were allowed to rest for 5 min between two consecutive trials. Swimming activity was monitored using a video camera mounted overhead. The latency time and traveled distance to escape onto the hidden platform were recorded by video tracking software. Memory retention was evaluated by a probe trial on day 35. In the probe trial, the platform was removed from the water maze apparatus and the animal was allowed to swim freely in the pool for 30 s. The swim speed, the number of crossings of the platform location and the time spent in the northern quadrant where the platform was previously located were recorded.

Novel object recognition

The novel object recognition test has been extensively used to evaluate the visuospatial memory of animals in a familiar environment [18, 19]. This memory test is based on the animal's natural propensity to explore novel objects. On day 32 and day 33, the animals were individu-

ally placed in the chamber (a perspex box with dimensions 80 × 60 × 50 cm) to habituate to the test environment for 10 min without any objects to explore. On day 34, two identical objects (O1 and O2) were placed in the chamber and the rats were allowed to freely explore the two objects for 5 min. The exploration test was repeated on day 35, with a familiar object (O3) and one novel different object (N) to explore. Time spent on exploring each object was recorded. The exploratory preference of the novel object was calculated as the ratio of the time spent on exploring the novel object (T_N) over that spent on the two objects ($T_N + T_{O3}$). Exploratory preference (%) = $T_N/(T_N + T_{O3}) \times 100$.

Measurements of Th17/Treg cytokines

Immediately after the sacrifice of the animal on day 35, the cortex of the brain and the left hippocampus were removed, homogenized in 4°C saline and centrifuged at 3000 rpm for 15 min to obtain the liquid supernatant. Levels of cytokines IL-17A, IL-23, TGF-β and IL-35 in homogenates were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the protocols provided by the manufacturer (CUSABIO, Wuhan, China). The samples were analyzed in duplicate and the mean values of the concentrations were used for statistical analysis.

Quantification of mRNA of RORγt and Foxp3 in brain

The right hippocampus and a small part of the brain cortex were removed immediately after sacrifice of the animal. Total RNA was extracted using Trizol Reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's instruction. Five µg of RNA was reversely transcribed using Prime Script RT Kit (TaKaRa, Dalian, China) in a final volume of 10 ml. The expression levels of RORγt and Foxp3 were detected by real-time RT-PCR using SYBR Green PCR Master Mix and ABI Prism 7500 Sequence Detection System (Applied Biosystems). Primer sequences were as follows: FoxP3: forward 5'-CTTCAGACAGCTTGTTTGCTG-3' reverse 5'-GGGCCGCATATTATGGTAC-TTG-3'; RORγt: forward 5'-CTGCACTGTGTGAAGGGTGA-3', reverse 5'-GACAAGCCTTTTCTCCATCG-3', GAPDH: forward 5'-GGCACAGTCAAGGCTGAGAATG-3', reverse 5'-ATGG ATGGTGGTGAAGACGCCAGTA-3'. The cycling conditions used were as follows: initial denaturation at 95°C for 30 s, followed by 35 cycles with denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and elongation at 72°C for 15 s. The expression level of GAPDH was used for internal control.

Statistical analysis

All data were presented as means ± SD. We used one-way ANOVA with a Tukey-Kramer test to determine the significance of differences in multiple comparisons. Differences with a p value of less than 0.05 were considered to be statistically significant.

Results

Effects of matrine on cognitive ability of rats in Morris water maze test

Spatial learning ability

We evaluated spatial learning ability of the animals in a water maze test. Figures 1 and 2 show that there were significant increases in latency time and traveled distance spent finding the invisible platform on day 30 to 34 in the AD group, if compared to the control and sham rats (all $p < 0.05$), suggesting the spatial learning impairment of the AD animals. Matrine (100 and 150 mg/kg/day) significantly reduced the latency time and the traveled distance of the animals on day 31 to 34, if compared to the AD group (both $p < 0.05$).

Memory retention

We evaluated memory retention of the rats by a single probe trial in the Morris water maze apparatus, in which the platform was removed. Time spent in the target quad-

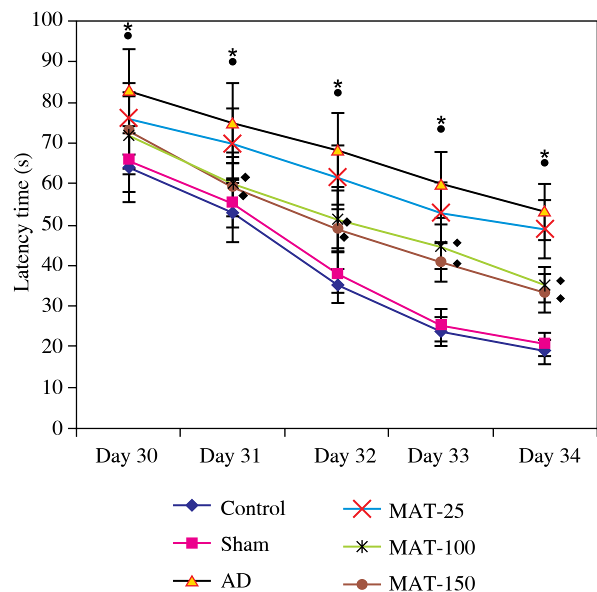

Fig. 1. Latency time of the rat in Morris water maze test. Spatial learning was tested from day 30 to 34 with 4 trials in a day. The mean latency time of the 4 trials represents the time spent finding the invisible platform placed in the fixed quadrant in the water maze apparatus. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (*$p < 0.05$, vs. control group; •$p < 0.05$, vs. sham group; ◆$p < 0.05$, vs. AD group)

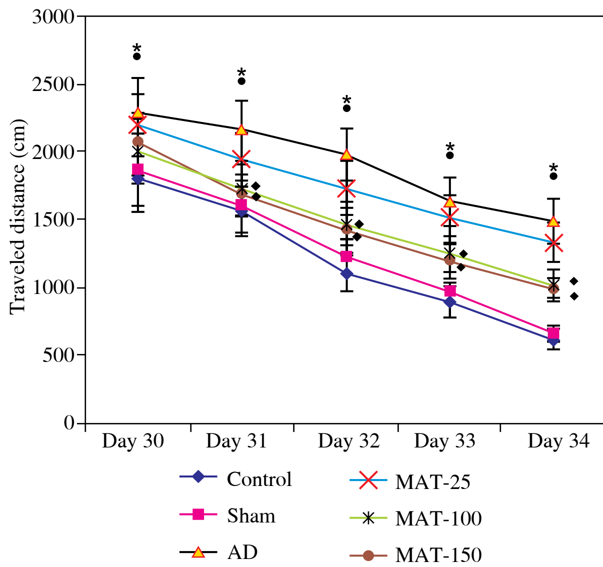

Fig. 2. Traveled distance of the rat in Morris water maze test. Spatial learning was tested from day 30 to 34 with 4 trials in a day. The mean traveled distance of the 4 trials represents the distance traveled to find the invisible platform placed in the fixed quadrant in the water maze apparatus. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (* $p < 0.05$, vs. control group; • $p < 0.05$, vs. sham group; ♦ $p < 0.05$, vs. AD group)

rant and platform-cross numbers of the AD group significantly decreased if compared to the control group and sham group (all $p < 0.05$), indicating less memory retention of the platform location than normal and sham groups. Matrine (100 and 150 mg/kg/day) significantly increased the time spent in the target quadrant and platform-cross numbers, if compared to the AD group (both $p < 0.05$), suggesting the improvement of the memory retention in the AD rats by MAT. The swim speeds of the groups were similar (all $p > 0.05$) (Figs. 3-5).

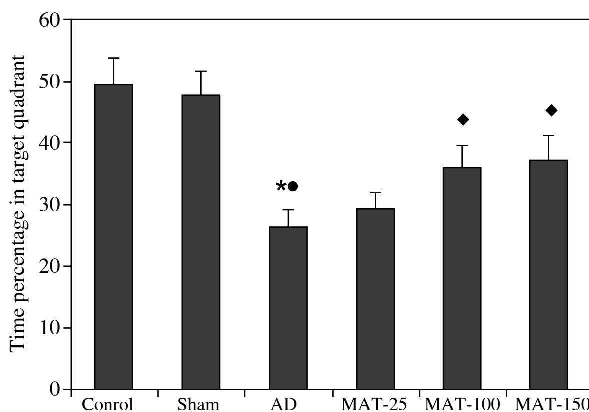

Fig. 3. Time spent in target quadrant of the rats in Morris water maze test. Memory retention was evaluated by a probe trial on the day 35. The bar represents the percentage of the time spent in target quadrant. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (* $p < 0.05$, vs. control group; • $p < 0.05$, vs. sham group; ♦ $p < 0.05$, vs. AD group)

Effects of matrine on cognitive ability of rats in a novel object recognition test

In a novel object recognition test, rats in the AD group spent less time exploring the novel object than the control and sham rats (both $p < 0.05$), suggesting distinct impaired memory in the AD rats. Yet, MAT (100 and 150 mg/kg/day) significantly prolonged the time spent exploring the novel object if compared to the AD group (both $p < 0.05$), indicating that MAT improved the visuospatial memory ability of the Aβ_{1-42}-induced AD rats (Fig. 6).

Effects of matrine on levels of Th17/Treg cytokines

Aβ_{1-42} injection significantly elevated levels of IL-17A and IL-23, but reduced levels of TGF-β and IL-35 in homogenates of the brain cortex and hippocampus in the AD group compared to the control and the sham groups (all $p < 0.05$), suggesting the imbalance of Th17/Treg cytokines in AD rats. However, MAT (100 and 150 mg/kg/day) significantly reversed the alterations in levels of IL-17A, IL-23, TGF-β and IL-35 if compared to the AD group (all $p < 0.05$) (Figs. 7 and 8).

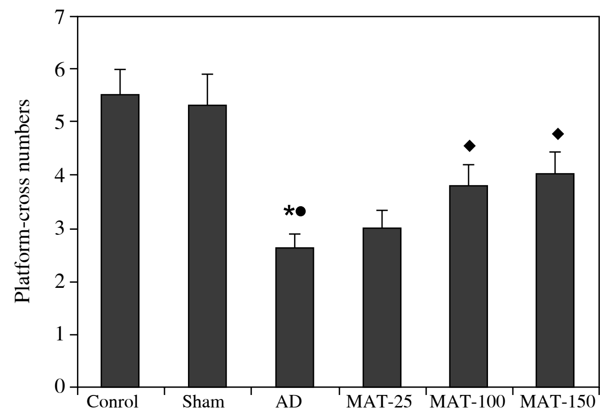

Fig. 4. Number of crossings of the platform location in Morris water maze test. Memory retention was evaluated by a probe trial on the day 35. The bar represents the number of crossings of the platform location. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (* $p < 0.05$, vs. control group; • $p < 0.05$, vs. sham group; ♦ $p < 0.05$, vs. AD group)

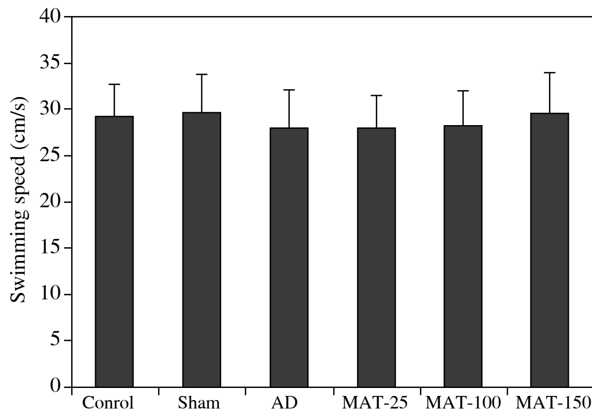

Fig. 5. Swimming speed of the rat in Morris water maze test. The bar represents the swimming speed of the rat in the probe trial detected on the day 35. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (*$p < 0.05$, vs. control group; •$p < 0.05$, vs. sham group; ◆$p < 0.05$, vs. AD group)

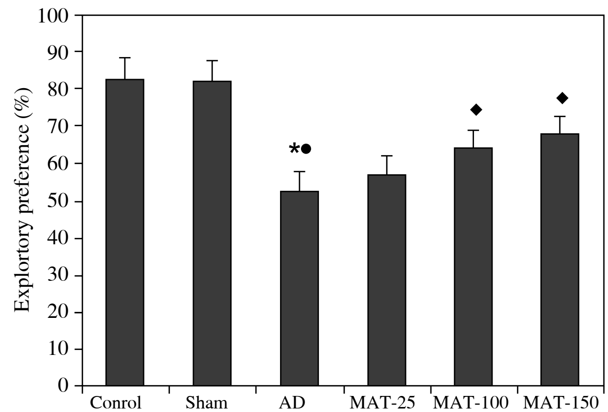

Fig. 6. Exploratory preference of the novel object in novel object recognition. The bar represents the exploratory preference of the novel object in novel object recognition test detected on day 35. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (*$p < 0.05$, vs. control group; •$p < 0.05$, vs. sham group; ◆$p < 0.05$, vs. AD group)

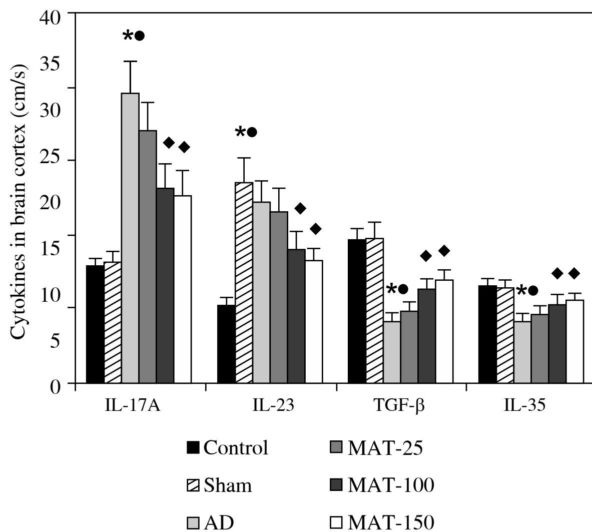

Fig. 7. Cytokine levels in brain cortex of the rats. Levels of cytokines in the homogenate of the brain cortex were measured by ELISA. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (*$p < 0.05$, vs. control group; •$p < 0.05$, vs. sham group; ◆$p < 0.05$, vs. AD group)

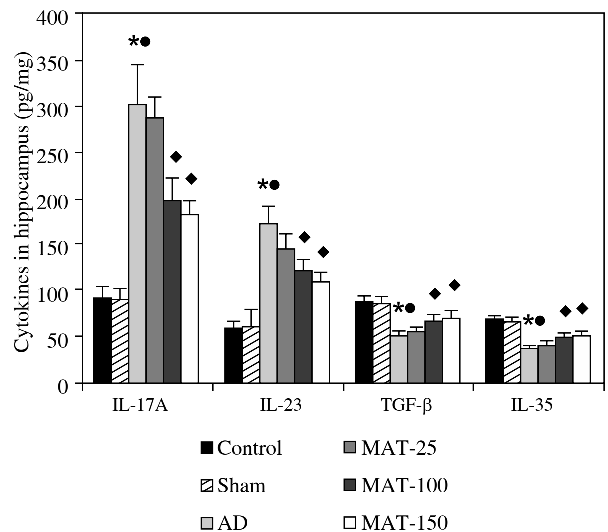

Fig. 8. Cytokine levels in hippocampus of the rats. Levels of cytokines in the homogenate of the hippocampus were measured by ELISA. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (*$p < 0.05$, vs. control group; •$p < 0.05$, vs. sham group; ◆$p < 0.05$, vs. AD group)

Effects of matrine on RORγt and Foxp3 mRNA expression

As shown in Figure 9 and 10, there was more mRNA expression of Th17 cell specific transcription factor RORγt but less mRNA expression of Treg cell specific transcription factor Foxp3 in the brain cortex and hippocampus of the AD group than the control group and the sham group (all $p < 0.05$). Within the 5 weeks of MAT treatment, the changes of RORγt and Foxp3 mRNA were significantly reversed in the MAT-100 group and the MAT-150 group, if compared to the AD group (both $p < 0.05$).

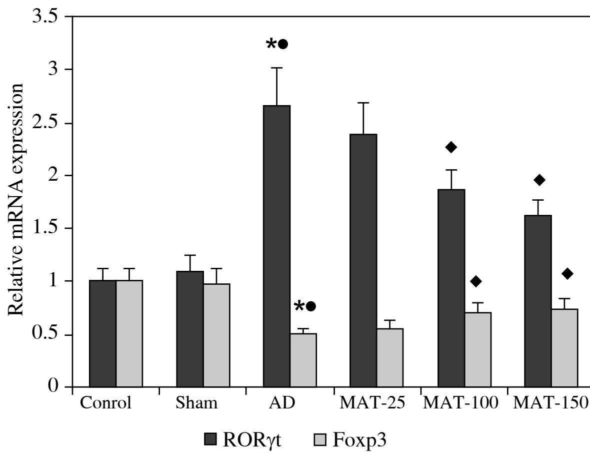

Fig. 9. Relative Foxp3 and RORγ mRNA expression in hippocampus. Foxp3 and RORγt mRNA expressions in hippocampus were measured by real-time RT-PCR. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (* $p < 0.05$, vs. control group; • $p < 0.05$, vs. sham group; ◆ $p < 0.05$, vs. AD group)

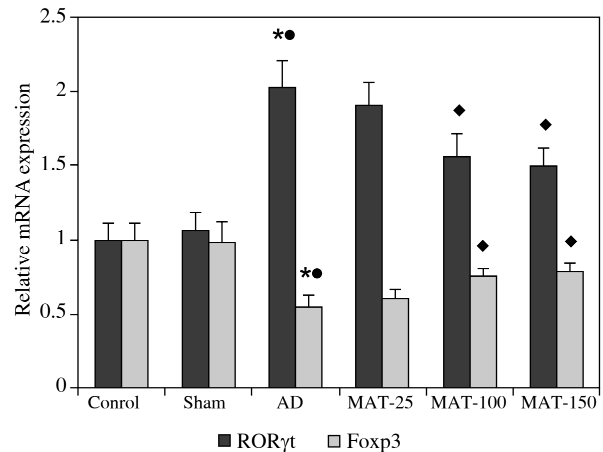

Fig. 10. Relative Foxp3 and RORγ mRNA expression in brain cortex. Foxp3 and RORγt mRNA expressions in brain cortex were measured by real-time RT-PCR. Data (means ± SD) were compared by one-way ANOVA following a Tukey-Kramer test to determine the significance of differences (* $p < 0.05$, vs. control group; • $p < 0.05$, vs. sham group; ◆ $p < 0.05$, vs. AD group)

Discussion

Alzheimer's disease is an aging-associated disorder and the most common form of dementia. Millions of AD patients suffer from the cognitive impairment. Food and Drug Administration has approved acetylcholinesterase inhibitor donepezil (Aricept), rivastigmine tartrate (Exelon), galantamine hydrobromide (Razadyne), and non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor memantine hydrochloride (Namenda) for the treatment of AD [20]. Yet, the current therapeutic effect is not satisfactory. Therefore, seeking new anti-AD agents is important.

Neuro-inflammatory process is associated with most neurodegenerative diseases [21]. It has been demonstrated that accumulation and aggregation of Aβ peptide in the hippocampus results in the activation of glial cells which, in turn, initiates a neuroinflammatory response, involving inflammatory cytokines. And the over-production of inflammatory cytokines may further activate the glial cells. In the pathogenesis of AD, the activation of glial cells and the release of proinflammatory mediators are considered to play a critical role. $A\beta_{1-42}$ induced AD animal model was extensively used in literature [22-24]. Injection of $A\beta_{1-42}$ to the hippocampus of animals could induce the activation of glial cells and over-production of inflammatory cytokines in hippocampus, thus leading to cognitive impairment [10, 25, 26]. Some agents that can inhibit the over-production of inflammatory cytokines improved AD in some animal studies. For examples, Zhang *et al.* [23] reported that atorvastatin attenuated the damage of nerve cells and improve learning and memory ability by inhibiting the production of IL-1β, IL-6, and TNF-α in the hippocampus of $A\beta_{1-42}$-induced rat model of AD; in a recent study, Sachdeva concluded that the amelioration of $A\beta_{1-42}$-induced spatial learning and memory impairment by lycopene in the AD rat model could be linked to the inhibition of nuclear factor kappaB (NF-κB) activity and the down-regulation of expression of neuroinflammatory cytokines [27]; there are some other studies that link the improvement of the cognitive impairment and the attenuation of neuroinflammation in $A\beta_{1-42}$-induced AD animals [28, 29]. Thus, anti-neuroinflammation management is now generally regarded as a potential strategy in treating AD.

The roles of cytokines IL-1β, IL-6, and TNF-α in the pathogenesis of AD have been well demonstrated. Recently, Th17 cell mediated inflammatory response is increasingly recognized as a contributor to the pathogenesis of AD [9, 10, 30]. As a new subset of T cell, Th17 cell mainly exerts its functions via its cytokines IL-17, IL-21, and IL-23 [7]. In the present study, we injected $A\beta_{1-42}$ to the hippocampus of the rats, which is a well-established method to induce AD in animals. Five weeks after the $A\beta_{1-42}$ injection, we detected levels of Th17 cell cytokines using ELISA and found levels of IL-17A, and IL-23 in the homogenates of the brain cortex and hippocampus significantly elevated compared to the rats without $A\beta_{1-42}$ injection. Our results were in line with the studies that reported the elevated levels of Th17 cytokines in AD patients and AD animals [9, 10, 12, 13, 31]. RORγt is the main transcription factor of Th17 cell. In the present study, we also measured the expression of RORγt mRNA. The re-

sults showed elevated levels of RORγt mRNA in the brain cortex and hippocampus. Consistently, two recent studies reported an increased expression of RORγt in AD [10, 22]. The alternations of transcription factor RORγt and Th17 cell cytokines in our study suggested the activation of Th17 cell in the brain of the AD rats. This is consistent with the other recent studies [9, 10].

Another kind of T cell involved in AD is the Treg cell. It is generally accepted that the Treg cell has immunosuppressive functions and plays a fundamental importance in modulating the balance between inflammation and immune tolerance [32]. Treg cell can secrets and exerts it functions via cytokines TGF-β and IL-35. In the present study, levels of cytokines TGF-β and IL-35 as well as Treg cell transcription factor Foxp3 mRNA in the brain cortex and hippocampus of the AD group significantly decreased in comparison with the control animals. The findings indicated that Treg cell was suppressed in the AD animal. Consistently, several studies suggested the role of Treg cell and its cytokines in AD. A recent study showed that the adoptive transference of Treg cells attenuated the cognitive impairment, Aβ-deposits and microglial activation in AD mice [33]. Transforming growth factor β1 were proved to have anti-neuroinflammation and anti-neurodegeneration activity in Aβ_{1-42}-induced AD animal models [12, 13]. All the findings suggested the protective role of Treg cell in AD.

The above results show the evident imbalance of Th17/Treg cytokines in the AD animal brain. In order to observe the effects of MAT on the production of Th17/Treg cytokines and transcription factors, we treated 3 groups of the animals with Aβ_{1-42} injection for 5 weeks. Our investigation showed that MAT (100 and 150 mg/kg/day, but not 25 mg/kg/day) markedly inhibited the overproduction of Th17 cytokines IL-17A and IL-23 as well as transcription factor RORγt in the brain cortex and hippocampus. However, MAT (100 and 150 mg/kg/day, but not 25 mg/kg/day) also significantly restored levels of Treg cytokines TGF-β and IL-35 as well as transcription factor Foxp3. The outcomes suggested that MAT could restore the balance of Th17/Treg cytokines in AD rats. Besides our findings, MAT has been proved to have anti-inflammatory activities by regulating Th2 cytokine production and some other mechanisms [34-38]. But to our knowledge, this is the first time that reports that MAT regulate the balance between Th17 cytokines and Treg cytokines in AD rats.

In addition to investigating the modulating effects of MAT on the production of Th17/Treg cytokines, we also determined its effects on the cognitive impairment in the AD rats. Morris water maze investigation which can be used to monitor the acquisition of spatial learning and memory of animals is one of the most commonly used methods to examine the cognitive function [39]. In the spatial learning trial, the rats of the AD group showed significant spatial learning impairment by presenting longer latency time and traveled distance to the invisible platform compared with the control animals and sham animals. In the probe trial which checked memory retention of spatial learning, the time spent in the target quadrant and the platform-cross numbers of rats in AD group also significantly decreased, indicating less memory retention of platform location than normal and sham groups. The outcomes of the Morris water maze test revealed the cognitive impairment of the AD rats. Yet, MAT improved the performance of the AD animals in the spatial learning trial and the probe trial at the dose of 100 and 150 mg/kg/day, but not at 25 mg/kg/day. The result indicates MAT could dose-dependently improve the learning and memory ability in the Aβ_{1-42}-induced AD rats. In order to further confirm the amelioration of cognitive impairment by MAT, we exposed the rats to a novel object recognition test which is a behavioral test commonly used to evaluate the visuospatial memory of animals in a familiar environment [18]. This memory test is based on the animal's natural propensity of exploring novel objects. The results showed that rats in the AD group spent less time exploring the novel object than the control and sham rats, suggesting distinct impaired memory in the AD rats. Yet, MAT (100 and 150 mg/kg/day, but not 25 mg/kg/day) significantly prolonged the time spent exploring the novel object, indicating that MAT improved the visuospatial memory ability of the Aβ_{1-42}-induced AD rats. Thus, both the Morris water maze investigation and the novel object recognition test consistently suggested the attenuation of the impairment of learning and memory by MAT treatment. Actually, the neuroprotective effects of MAT were already studied in some studies. Kan reported that MAT protected neuro-axon from CNS inflammation-induced injury [40]. Zhang reported the inhibitory effect of MAT on blood-brain barrier disruption for the treatment of experimental autoimmune encephalomyelitis [41]. Xu found that MAT had neuroprotective effects against focal cerebral ischemia and directly protects neurons and astrocytes via inhibition of NF-κB signaling pathway [42]. But to our knowledge, this is the first time that reports that MAT attenuates the cognitive impairment in AD rats.

Overall, the results demonstrated that the AD rats had imbalance of Th17/Treg cytokines in brain. MAT could dose-dependently restore the relative balance of Th17/Treg cytokines and attenuate the cognitive impairment in AD rats. The limitations of our study are that we only investigated the Aβ_{1-42}-induced AD animal model, and we mainly focused on the mechanism of the imbalance between Th17/Treg cytokines. The other possible mechanisms of improving the cognitive function and the effects in other AD model even in other neurodegenerative disorders should be addressed in future studies.

The authors declare no conflict of interest.

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