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A combination of indomethacin and atorvastatin ameliorates cognitive and pathological deterioration in PrP-hAβPPswe/PS1$^{\Delta E9}$ transgenic mice

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ABSTRACT

Mounting evidence has shown that inflammation might drive Alzheimer's disease (AD) pathology and contribute to its exacerbation. Previous studies have indicated that indomethacin or atorvastatin are beneficial in treating AD; however, no significant clinical effects have been shown. Furthermore, no study has investigated the efficacy of combining these agents for treating AD. This study sought to determine the effect of a combination of indomethacin and atorvastatin in the PrP-hAβPPswe/PS1$^{\Delta E9}$ (APP/PS1) transgenic AD mouse model. Treatment with indomethacin and atorvastatin ameliorated impairments in spatial learning and memory, and the active avoidance response in APP/PS1 mice. Moreover, we found a suppression of Aβ plaques and decreased concentration of $A\beta_{1-42}$ in the hippocampus of APP/PS1 mice following treatment. In addition, indomethacin and atorvastatin ameliorated abnormal cytokine secretion, lymphocyte subset disorder, and hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axis imbalances in APP/PS1 mice. The combination of indomethacin and atorvastatin restored immune and neuroendocrine processes, attenuated pathologic changes and cognitive impairments in APP/PS1 transgenic mice, and could thus be a potential therapeutic agent for AD.

1. Introduction

Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disorder, which represents the most common cause of dementia worldwide. All current approved first-line pharmacological treatments for AD treat the symptoms of AD, but do not delay AD progression. Emerging evidence suggests that inflammation has a causal role in AD pathogenesis. Neuroinflammation is not a passive system activated by emerging Aβ and neurofibrillary tangles, but instead contributes as much or more to pathogenesis as these AD hallmarks (Zhang et al., 2013a). This indicates that neuroinflammation could be a key mechanism in AD. In addition, development and exacerbation of cognitive decline after a peripheral inflammatory challenge, such as an infection or an aseptic injury (Kyrkanides et al., 2011), shows the communication between acute and chronic systemic inflammation and brain pathology. Therefore, understanding and controlling interactions between the immune and nervous system might be key to the prevention or delay of AD progression.

Epidemiological, clinical, and neuropathological studies have shown that long-term treatment with indomethacin, a nonselective COX-1 inhibitor, ameliorates cognitive dysfunction or slows cognitive decline with a concomitant reduction in Aβ plaques(Rogers et al., 1993; Weggen et al., 2001). Moreover, indomethacin is effective in different experimental models of cerebral inflammation and AD mouse models (Boehme et al., 2014).

An altered cholesterol metabolism is seen in AD, de novo of cerebral cholesterol synthesis is decreased in AD (Kölsch et al., 2010). These findings have raised doubt regarding the beneficial effect of cholesterol-lowering therapies in AD (Zhang et al., 2013b). Data from animal model studies have indicated that atorvastatin may prevent AD, reducing Aβ, β-secretase (BACE1) protein expression, and oxidative stress (Kurata et al., 2011; Murphy et al., 2010), whereas other statins have shown no difference (Feldman et al., 2010). Hence, atorvastatin may be beneficial in AD by suppressing inflammation.

A number of studies demonstrate disturbances in the central innate immune system in patients with AD and an exaggerated central innate immune response following systemic inflammation in animal models of neurodegeneration (C, 2011). Therefore, we hypothesized that long-term treatment with a combination of indomethacin and atorvastatin could ameliorate cognitive deterioration in APP/PS1 transgenic mice

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via suppression of COX-1 and HMG-CoA reductase.

2. Materials and methods

2.1. Experimental animals

Adult male 9-month-old heterozygous APPswe/PS1^{dE9} (APP/PS1) transgenic mice and wild type (WT) nontransgenic littermates were obtained from Beijing HFK Bioscience Co., Ltd., via The Jackson Laboratory (Bar Harbor, ME, USA). APP/PS1 mice expresses chimeric amyloid precursor protein (APPswe) encoding the Swedish mutations K595N/M596L, and human presenilin 1 PS1-dE9controlled by independent mouse prion protein promoter elements. Mice were maintained at the Beijing Institute of Pharmacology and Toxicology under standard housing conditions. The animal treatment, husbandry, and experimental protocols of the present study received approval from the Institute of Animal Care and Use Committee (IACUC) of the National Beijing Center for Drug Safety Evaluation and Research (NBCDSER).

APP/PS1 transgenic mice were randomly separated into 4 groups (n = 10–11 per group). Indomethacin (0.25 mg/mL; Ouhe Chemical Ltd., Beijing, China), atorvastatin (0.2 mg/mL; Ouhe Chemical Ltd., Beijing, China, memantine (1 mg/mL; Ouhe Chemical Ltd., Beijing, China), and donepezil (0.1 mg/mL; Ouhe Chemical Ltd., Beijing, China) were dissolved in 40% PEG400. Drugs were administered via intragastric administration (0.1 mL/10 g body weight) once a day for 150 days. One group of APP/PS1 transgenic mice (model, n = 11) and age-matched WT mice (control, n = 15) were administered an equal volume of deionized water with 40% PEG400. Drug administration and behavioral tests were conducted according to the experimental timelines shown in Fig. 1. Following the behavioral experiments, mice were placed in a sealed chamber and euthanized via isoflurane inhalation and cervical dislocation.

2.2. Behavioral tests

2.2.1. Morris water maze

The Morris water maze was performed as described in Vorhees and Williams (Vorhees and Williams, 2006). The apparatus of Morris-water maze test consisted of a 90 cm in diameter and 45 cm wall height circular pool with a black inner surfer, placed in a dim light sound-proof room. The pool was divided into 4 quadrants, a 6 cm in diameter black platform in the first quadrant. Briefly, the spatial learning phase consisted of 4 trials per day for 5 days. The next day, the probe trial was performed. Mice were placed on the platform for 60 s before the first trial, and then released into the water to search for the platform within 60 s. If the mouse found the platform within 60 s, it was allowed to remain for 5 s. If the mouse did not find the platform within 60 s, it was gently led to the platform and allowed to remain for 5 s. In the spatial memory phase, the platform was removed, and the mouse was placed into the water at a novel position and allowed to swim freely for 60 s. Spatial memory was assessed by recording the time spent in the target quadrant and number of platform crossings.

2.3. Shuttle box test

The shuttle box test was performed as described in Cheng et al. (Cheng et al., 2011). Working memory was evaluated with a shuttle box apparatus (VT 05448, Med Associates Inc., East Fairfield, VT, USA). The training session began with acclimatization for 2 min, and followed by 30 trials, with an inter-trial interval of 15 s. A tone (60 dB) and light (8 W) were presented as the conditioned stimulus (CS) for 10 s, followed by 5 s of electrical foot shock (0.2 mA) as the unconditioned stimulus (US). If the mouse moved to the opposite chamber during presentation of the CS, no US was presented and an active avoidance response was recorded. The shuttle-box procedure was performed for 5 days. On day 6, all the mice underwent an additional session with no US to assess conditional memory, and the number of active avoidance response was recorded.

2.4. Biochemical and histochemical analyses

2.4.1. Immunofluorescence

Brains were removed and one hemisphere was fixed via immersion in 4% paraformaldehyde, and then paraffin embedded. Serial 5-μm-thick sections were prepared. Following this, sections were incubated with mouse anti-β-amyloid antibody (6E10, 1:100, Biolegend, CA, USA) overnight at 4 °C. The sections were incubated with goat anti-mouse IgG HRP (1: 1000, ZSGB-Bio, Beijing, China) for 2 h at 37 °C, then incubated using an Opal 520 reagent pack (PerkinElmer, Waltham, MA, USA) according to manufacturer's instructions. Tissue sections were photographed with a fluorescence lifetime imaging microscope (Vectra 2, PerkinElmer-Caliper LS, Waltham, MA, USA).

2.5. Soluble Aβ analysis

The Aβ AlphaLISA assay was carried out as described in Cheng et al. (Cheng et al., 2013). The $A\beta_{1-40}$ and $A\beta_{1-42}$ concentration in the hippocampus, cortex, and plasma were determined using the human $A\beta_{1-40}$ (high specificity; AL275C, PerkinElmer, Waltham, MA, USA) and human $A\beta_{1-42}$ (high specificity; AL276C, PerkinElmer) kits according to the manufacturers' instructions.

2.6. Multiplex bead analysis

Plasma samples were analyzed using a Luminex 200™ (Luminex, Austin, TX, USA). The protein expression of IL-1β, IL-2, IL-5, IL-17, IL-6, IL-4, IL-10, GM-CSF, G-CSF, IFN-γ, TNF-α, MCP-1, RANTES, eotaxin, and MIP-1β were detected using a multiplex map kit (MCYTOMAG-70 K, Millipore). IL-23 and TNF-β protein expression was detected using another multiplex map kit (MGAMMAG-300 K, Millipore).

2.7. Flow cytometric analysis

Mouse spleen cells were harvested and divided into three parts. The following fluorochrome conjugated antibodies were used: anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CD28, anti-CD19, anti-CD80, anti-Foxp3 for flow cytometry (BioLegend). After incubation, the cells were

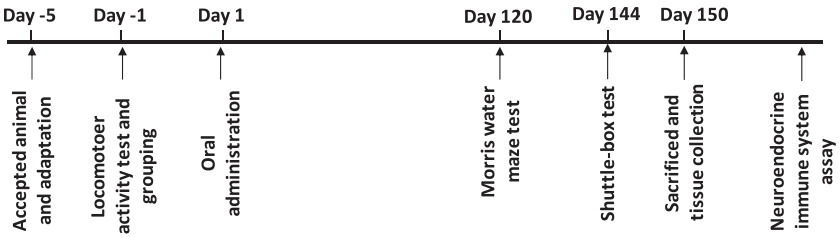

Fig. 1. The schematic diagram of experimental procedure.

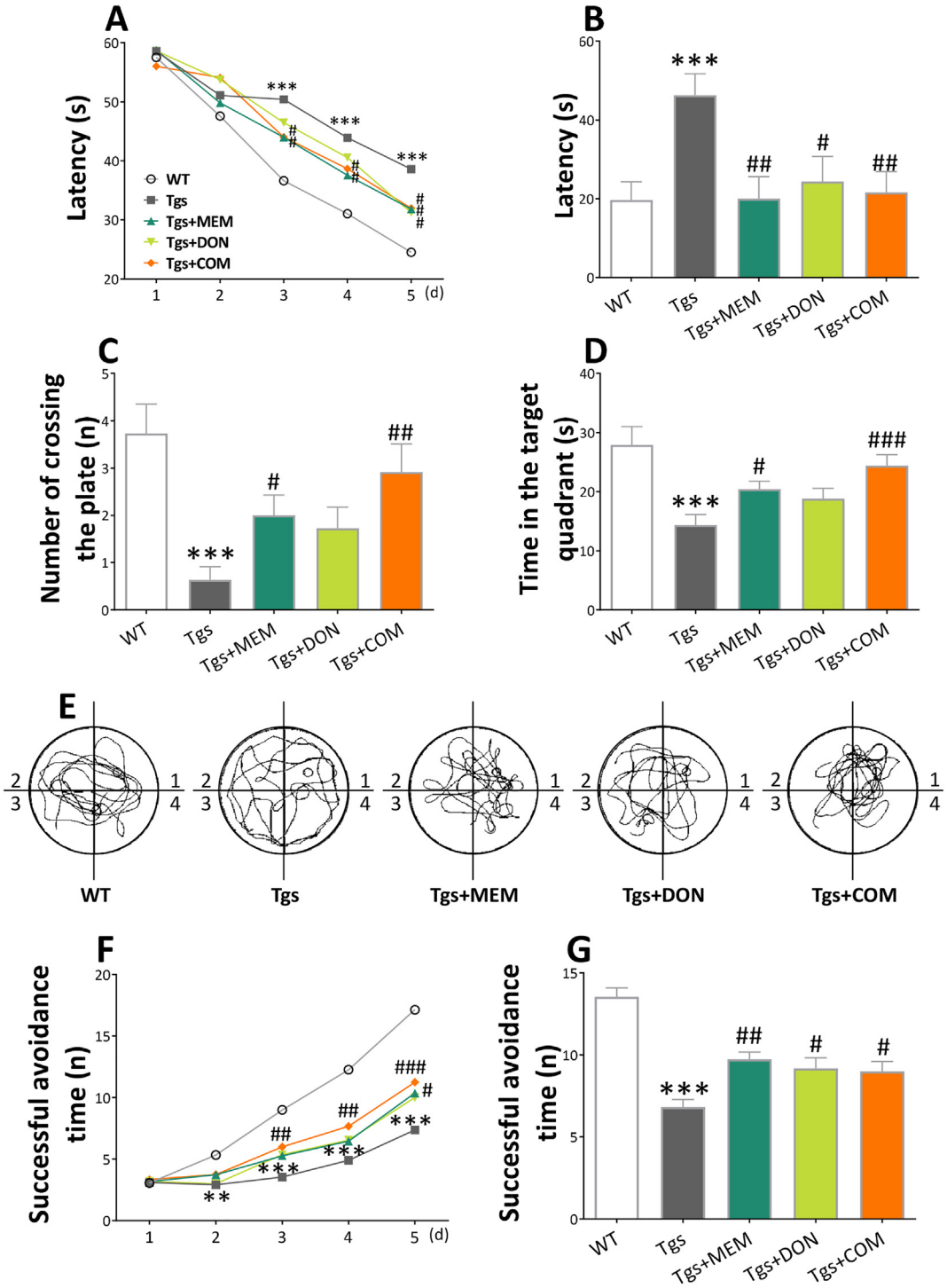

Fig. 2. Effect of combination with indomethacin and atorvastatin on the learning and memory in APP/PS1 mice. The Morris water maze test was used. (A) Escape latency, the first time that the mice found the hidden platform in the learning task. (B) Escape latency, the first time that the mice found the hidden platform in the place navigation test. (C) Numbers that the mice crossed the removed platform in the spatial probe trial. (D) The swimming time within the target quadrant in the spatial probe trial. (E) Track plots show the movement of mice in spatial probe trial. The shuttle-box test was used. The successful avoidance times in training (F) and testing (G) phase. The values are mean ± SEM. $n = 11–15$. $^{**}P < 0.01$, $^{***}P < 0.001$, versus the WT mice by unpaired Student's *t*-test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus the APP/PS1 mice, by one-way ANOVA analysis followed by Dunnett's post hoc test and two-way repeated-measures analysis of variance with Tukey multiple comparisons test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination.

washed and resuspended in 0.5 mL of PBS/2% paraformaldehyde, and then quantified using flow cytometry (BD Calibur ™ San Jose, CA, USA).

2.8. Radioimmunoassay of hypothalamic and hypophyseal hormones

The hypothalamus and pituitary gland were weighed and boiled. Peptides were extracted using the glacial acetic acid methods according to the manufacturer's protocol of radioimmunoassay kit. Hormone

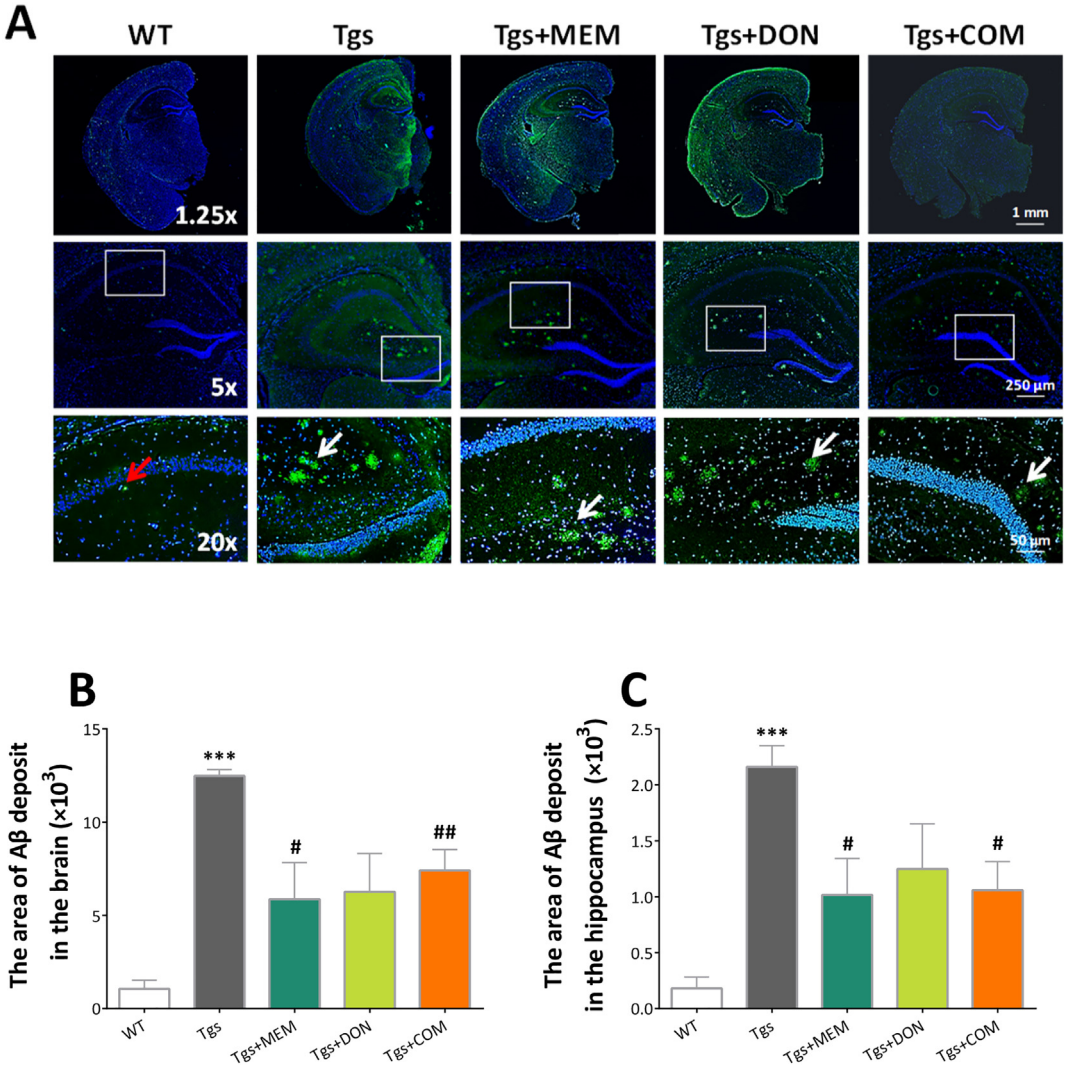

Fig. 3. Suppressive effects of combination with indomethacin and atorvastatin on APP/Aβ plaques in the brain of APP/PS1 mice. (A) Representative immunofluorescence staining images showing APP/Aβ plaques (green and indicated by white arrows) in the hippocampus and brain of WT and Tgs mice. Quantification of APP/Aβ plaques in the brain (B) and hippocampus (C) of WT and Tgs mice by Image Pro Plus 6.0 software. White arrows indicate APP/Aβ plaques in hippocampus, red arrows indicate false positive result of APP/Aβ plaques. The values are mean ± SEM. $n = 11–15$. $^{***}P < 0.001$, versus the WT mice by unpaired Student's *t*-test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus the Tgs mice by one-way ANOVA analysis followed by Dunnett's post hoc test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations were determined using ^{125}I-ATCH, ^{125}I-LH, ^{125}I-FSH RIA kits (North Institute of Biological Technology), ^{125}I-CRH and ^{125}I-GnRH RIA kits from the Department of Neurobiology, Second Medical University.

2.9. Enzyme-linked immunosorbent assay

The concentration of corticosterone (CORT) in the plasma was measured via ELISA (EC3001–1, ASSAYPRO, Charles, MO, USA) according to the manufacturer's instructions. The absorbance was measured at 450 nm with a reference wavelength of 570 nm using an Enspire™ multilabel reader 2300 (Perkin Elmer, Turku, Finland).

2.10. Immunochemiluminescence assay

Testosterone (T) was measured using an Access Immunoassay System (Beckman Coulter, Brea, CA, USA), access testosterone (33,560, Beckman Coulter) and access testosterone calibrators (33,565, Beckman Coulter). The entire measurement was automatically processed according to the scheduled program.

2.11. Statistical analysis

All data are expressed as mean ± SEM. GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) was used to plot and analyze the data. Data between two groups were compared using a Student's *t*-test. Comparison of data between multiple groups was performed using a one-way analysis of variance (ANOVA) followed by a Dunnett's post hoc test or a two-way repeated-measures ANOVA with post-hoc Tukey multiple comparisons test. Results were considered statistically significant when $P < 0.05$.

3. Results

3.1. Indomethacin and atorvastatin improved learning and memory in APP/PS1 mice

APP/PS1 transgenic mice had significantly longer escape latencies

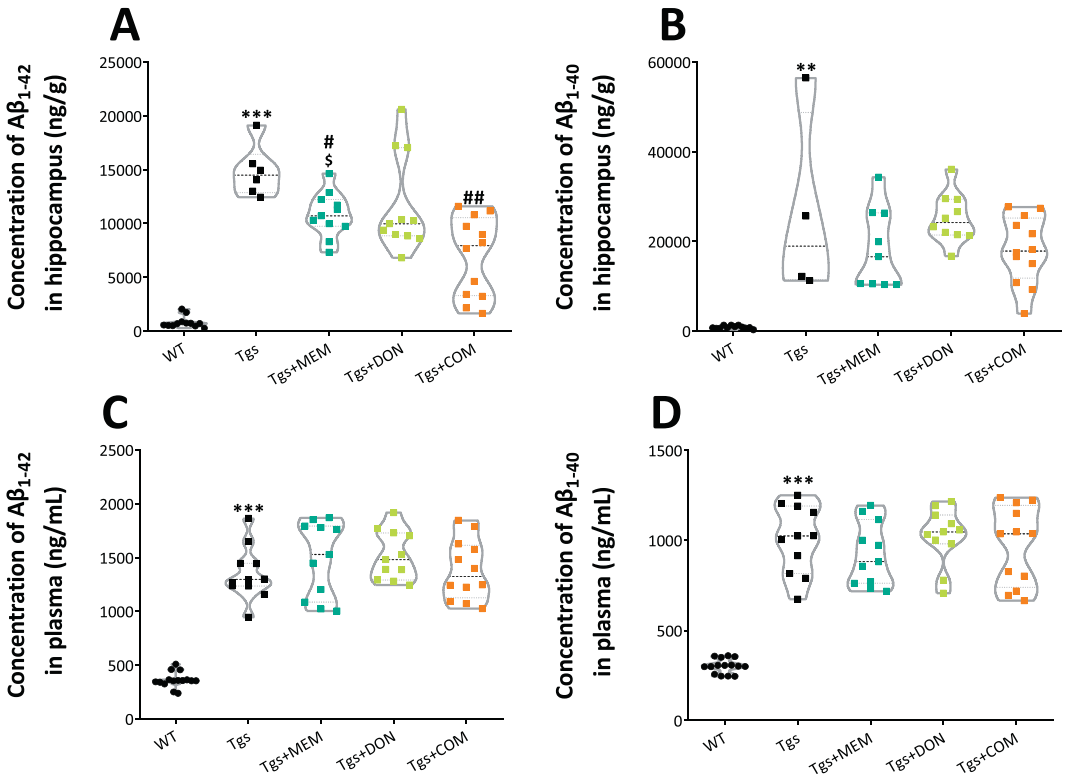

Fig. 4. Effect of combination with indomethacin and atorvastatin on the levels of Aβ in APP/PS1 mice. The concentration of $A\beta_{1-42}$ and $A\beta_{1-40}$ in brain (A and B) and plasma (C and D) of Tgs mice by AlphaLISA assay. The values are mean ± SEM. $n = 11–15$. $^{**}P < 0.01$, $^{***}P < 0.001$, versus the WT mice by unpaired Student's *t*-test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus the Tgs mice by one-way ANOVA analysis followed by Dunnett's post hoc test, $^{\$}P < 0.05$, versus combination group by Kruskal-Wallis test followed by Dunnett's post hoc test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination.

compared with WT mice on the last test day, and the latencies in the combination-, donepezil- or memantine-treated APP/PS1 transgenic mice were significantly longer than the control group (Fig. 2A). In the spatial memory phase, the mean escape latency was longer (Fig. 2B), the number of plate crossings diminished (Fig. 2C), and the time in the target quadrant was shorter (Fig. 2D); however, there was no significant difference in swimming speed (data not shown) in APP/PS1 transgenic mice compared with WT mice. Escape latencies were reduced in combination-, donepezil- or memantine-treated mice, and the number of plate crossings expanded and the time in the target quadrant was reversed in mice treated with combination and memantine. These findings show that administration of the combination of indomethacin and atorvastatin ameliorated spatial learning and memory deficiencies in APP/PS1 transgenic mice.

In the shuttle-box test, significantly fewer successful avoidance times were observed for APP/PS1 transgenic mice than for WT mice after the second day of the training phase, but significantly increased after treatment with combination, memantine, or donepezil after the third day (Fig. 2F). In the testing stage, there was a significantly decrease in avoidance times in APP/PS1 transgenic compared with WT mice; however, this was rescued after administration of combination, memantine, or donepezil treatments (Fig. 2G). These results demonstrate that there was a deterioration in the active avoidance response in APP/PS1 transgenic mice, which was ameliorated after treatment with a combination of indomethacin and atorvastatin.

3.2. Administration of indomethacin and atorvastatin alleviated Aβ level in APP/PS1 mice

Aβ plaques in the cerebrum is a pathological hallmark of AD in patients and animal models. Immunofluorescent staining of brain sections stably expressing human APP and Aβ with the antibody 6E10, which labels both APP and Aβ. Our results demonstrated that APP/PS1 transgenic mice developed a critical number of APP/Aβ plaques in the brain at 14 months, while APP/Aβ plaques were not found in WT mice (Fig. 3A). Combination- or memantine-treated mice showed a reduction in the area of APP/Aβ plaques in the entire cerebrum and hippocampus of APP/PS1 transgenic mice (Fig. 3B and C). These results demonstrate that APP/Aβ plaques in the brain of APP/PS1 transgenic mice was ameliorated after administration of the combination of indomethacin and atorvastatin. The AlphaLISA assay demonstrated that the concentration of $A\beta_{1-42}$ and $A\beta_{1-40}$ in the hippocampus and plasma of APP/PS1 transgenic mice was significantly higher than that in WT mice (Fig. 4A, B, C, and D). Combination treatment led to a significant decrease in the concentration of $A\beta_{1-42}$ in the hippocampus of APP/PS1 transgenic mice when compared with WT mice. Moreover, the $A\beta_{1-42}$ concentration in the hippocampus of combination-treated APP/PS1 transgenic mice was significantly lower than that in memantine-treated group. These data indicate that combination treatment with indomethacin and atorvastatin down-regulated $A\beta_{1-42}$ in the hippocampus of APP/PS1 transgenic mice, and this effect was greater than memantine treatment.

3.3. Indomethacin and atorvastatin treatment modulated abnormal cytokine secretion and lymphocyte subsets in APP/PS1 mice

There were increases in the concentration of interleukin (IL)-1β and IL-23, tumor necrosis factor (TNF)-α, and TNF-β, colony stimulating factor (GM-CSF), the chemotactic factor, eotaxin, and reductions in the concentration of IL-4 and G-CSF in the plasma were observed in APP/PS1 transgenic mice when compared with WT mice (Fig. 5). The concentration of IL-1β, IL-23, GM-CSF, TNF-α, TNF-β, and eotaxin was

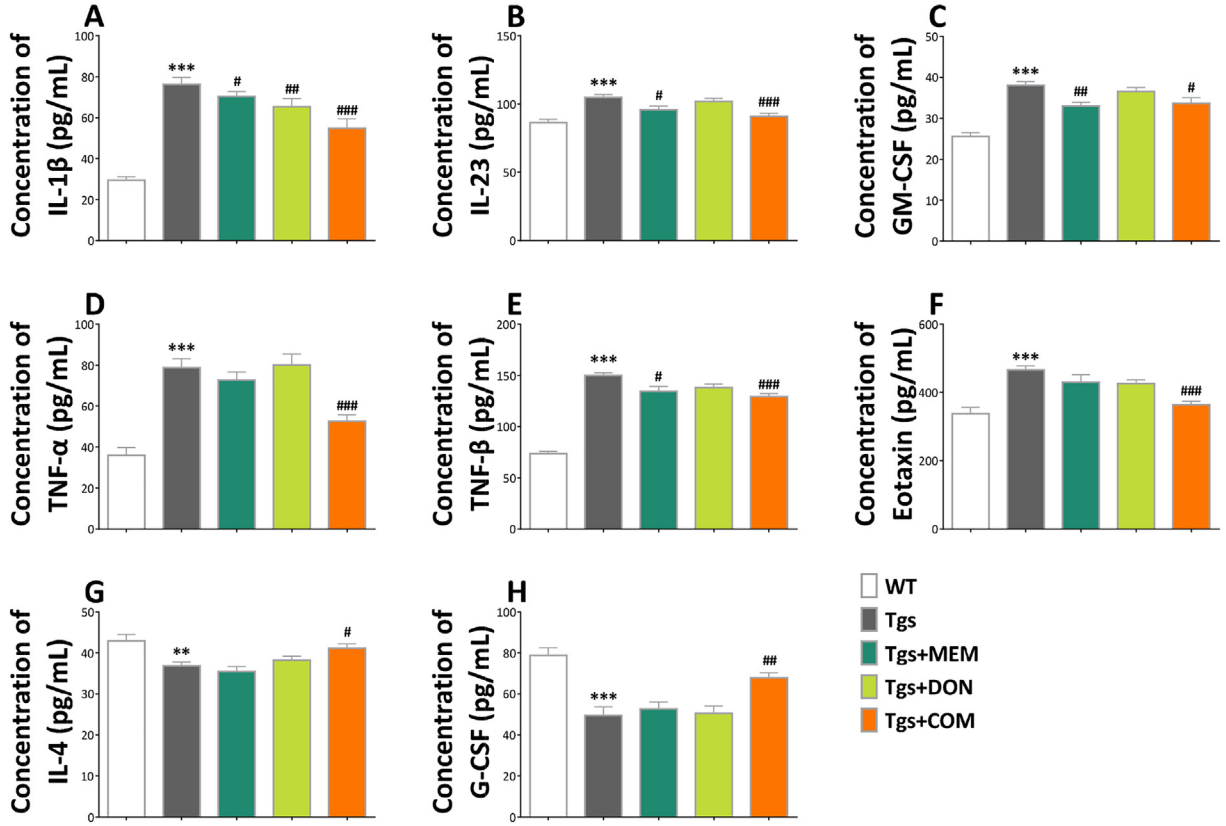

Fig. 5. Effect of combination with indomethacin and atorvastatin on the concentration of cytokines in the plasma of APP/PS1 mice. Concentrations (pg/mL) of (A) interleukin-1β (IL-1β), (B) interleukin-23 (IL-23), (C) granulocyte-macrophage colony stimulating factor (GM-CSF), (D) tumor necrosis factor α (TNF-α), (E) tumor necrosis factor β (TNF-β), (F) Eotaxin, (G) interleukin-4 (IL-4), and (H) granulocyte colony stimulating factor (G-CSF) in the blood plasma were detected using Luminex® X-MAP® technology. The values are mean ± SEM. $n = 11–15$. $^{**}P < 0.01$, $^{***}P < 0.001$, versus the WT mice by unpaired Student's *t*-test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus the Tgs mice by one-way ANOVA analysis followed by Dunnett's post hoc test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination.

decreased, IL-4 and G-CSF were elevated after a combination of indomethacin and atorvastatin treatment. These results show that cytokine secretion in APP/PS1 transgenic mice was irregular and administration of a combination of indomethacin and atorvastatin restored immune function in APP/PS1 transgenic mice.

There were significantly fewer $CD8^+$ $CD28^+$ T cells (Fig. 6A) and more $CD4^+$ $CD25^+Foxp3^+$ T cells in APP/PS1 transgenic mice than in WT mice (Fig. 6B). The expression of other lymphocyte subsets in APP/PS1 transgenic mice were not significantly different from WT. In APP/PS1 transgenic mice, the expression of $CD4^+$ $CD28^+$ T cells increased after treatment with memantine, donepezil, or combination, while the expression of $CD4^+CD25^+Foxp3^+$ T cells diminished after treatment with memantine or indomethacin and atorvastatin. These results demonstrate that combination treatment with indomethacin and atorvastatin partially restored lymphocyte expression in APP/PS1 transgenic mice.

3.4. Indomethacin and atorvastatin restored neuroendocrine hormone balance in APP/PS1 mice

To assess whether the combination with indomethacin and atorvastatin regulated the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes in APP/PS1 transgenic mice, the concentration of HPA and HPG hormones were measured. The concentration of CRH, ACTH, and CORT was significantly higher in APP/PS1 transgenic mice than in WT mice (Fig. 7A, B, and C). However, there was no significant difference in testosterone concentration (data not shown) in APP/PS1 transgenic mice compared with WT mice. The administration of the combination treatment and memantine significantly diminished the concentration of ACTH. The concentration of GnRH, FSH, and LH increased in APP/PS1 transgenic mice (Fig. 7D, E, and F). The combination treatment significantly decreased the concentration of GnRH and LH. These data show that the combination of indomethacin and atorvastatin ameliorates endocrine system irregularities in APP/PS1 transgenic mice, particularly in the HPA and HPG axes.

4. Discussion

Neuroinflammation has been a hallmark of AD since the disease was first described by Alzheimer (Alzheimer, 1907). Evidence indicates that neuroinflammation might drive the pathogenic process of AD and contribute to its exacerbation (Calsolaro and Edison, 2016). Excessive Aβ production and deposition alone might be sufficient to induce an inflammatory reaction that subsequently contributes to cognitive decline and the development of AD (Eikelenboom et al., 2012). The study here provided preliminary experimental evidence for the beneficial effect of combining indomethacin and atorvastatin for treating AD, and supported the potential effectiveness of combinatorial therapies for the possible attenuation of cognitive impairment and Aβ pathology in APP/PS1 transgenic mice.

Behavioral studies have suggested an inverse association between the use of indomethacin and risk of AD (Bentham et al., 2008; in t' Veld et al., 2001). Animal studies have shown that treatment with indomethacin significantly reduces hippocampal Aβ in Tg2576 mice (Sung et al., 2004). Additionally, indomethacin is the only NSAID shows anti-amyloid effects in AD animal models (Vlad et al., 2008). Administration of atorvastatin rescued learning and memory

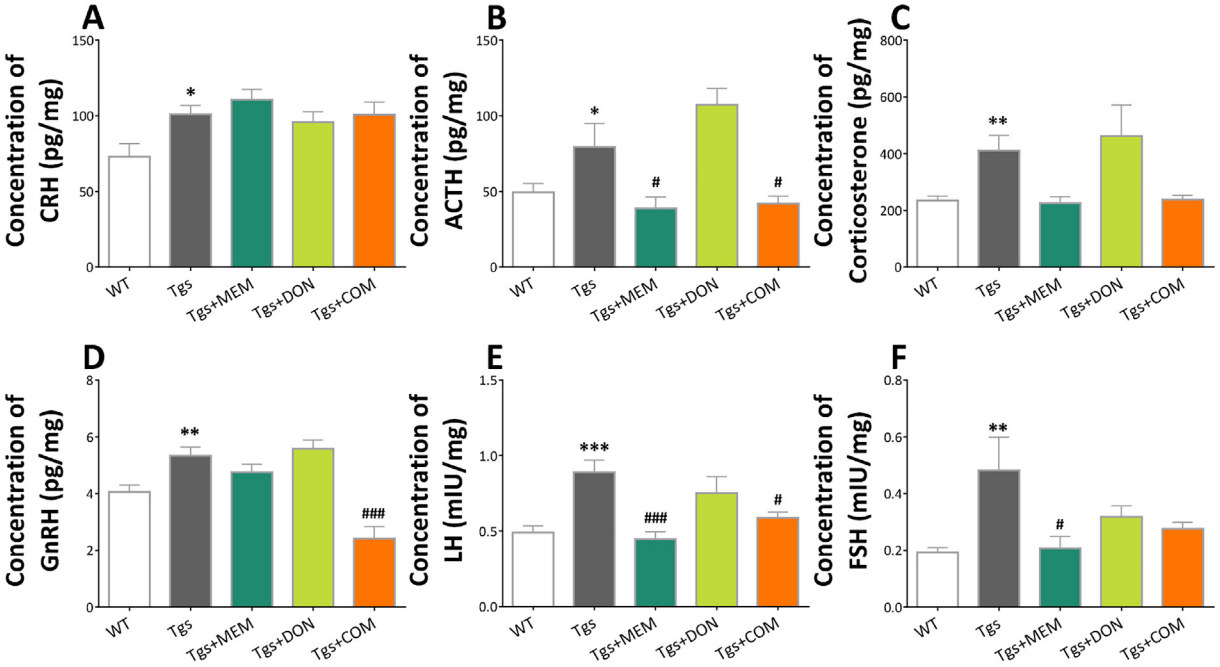

Fig. 6. Effect of combination with indomethacin and atorvastatin on the subsets of spleen lymphocytes in APP/PS1 mice. Flow cytometric analysis of $CD8^+$ $CD28^+$ T cells (A) and $CD4^+$ $CD25^+Foxp3^+$T cells (B) in the spleen supernatant of mice. 5×10^5 spleen cells isolated from mouse (n = 11–15) spleen were harvested, divided into three parts, washed, and followed by incubation with antibodies, then quantified by flow cytometry, the sample size of the flow cytometric analysis was three. The values are mean ± SEM. $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$, versus the WT mice by unpaired Student's t-test, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus the Tgs mice by one-way ANOVA analysis followed by Dunnett's post hoc test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination.

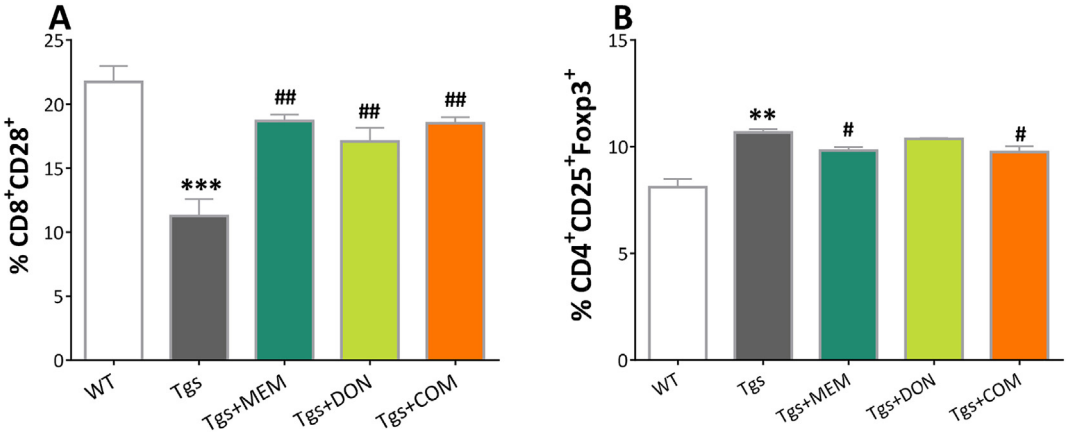

Fig. 7. Effect of combination with indomethacin and atorvastatin on the hormone secretion of HPA and HPG axis in APP/PS1 mice. The hormones of hypothalamic-pituitary-adrenal axis including (A) corticotropin releasing hormone (CRH) in hypothalamus, (B) adrenocorticotropic hormone (ACTH) in pituitary, and (C) corticosterone (CORT) in plasma. The hypothalamic-pituitary-gonadal axis including (D) gonadotropin-releasing hormone (GnRH) in hypothalamus, (E) follicle-stimulating hormone (FSH) in pituitary, and (F) luteinizing hormone (LH) in pituitary. The values are mean ± SEM. n = 11–15. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus the WT mice by unpaired Student's t-test, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$, versus the Tgs mice by one-way ANOVA analysis followed by Dunnett's post hoc test. WT means wild mice, Tgs means APP/PS1 mice, MEM means memantine, DON means donepezil, COM means combination.

impairments by inhibiting production of IL-1β, IL-6, and TNFα (Zhao et al., 2016). Furthermore, in line with the attenuation of cytokine production, such as IL-6, IL-1β and TNFα, which is reported in previous studies of indomethacin or atorvastatin, we found that the combination of indomethacin and atorvastatin modulated abnormal secretion of many cytokines and chemokines, including IL-23, GM-CSF, eotaxin, IL-4, and G-CSF. It is well known that IL-23, GM-CSF, eotaxin, IL-4, and G-CSF are associated with amyloid load and memory deterioration in AD. Moreover, our results showed that treatment with a combination of indomethacin and atorvastatin significantly ameliorated the abnormal expression of $CD8^+CD28^+$ and $CD4^+CD25^+Foxp3^+$ T-cells (Tregs) in APP/PS1 transgenic mice. Abnormal lymphocyte expression is shown in patients with AD when compared with healthy elderly individuals, and is enhanced by beta-amyloid peptides (Baruch et al., 2015). This indicated that the administration of indomethacin and atorvastatin provided unique benefits compared with indomethacin or atorvastatin treatment alone.

In addition, the neuroendocrine immunomodulation (NIM) network maintains the processes of homeostasis and defends against hostile environmental factors. Increasing literature shows that NIM network imbalance contributes to the pathogenesis of AD; therefore, rebalancing the NIM network could be beneficial in patients with AD (Reese and Taglialatela, 2010). In previous literature, the concentrations of ACTH and corticosterone in HPA axis (H et al., 2009; JH et al., 2016), GnRH,

FSH and LH in HPG axis (AP et al., 2017) were unaffected by donepezil administration. However, the effect of memantine on the levels of ACTH and corticosterone was ambiguous, such as the stress-induced increases in corticosterone levels were reversed by memantine (GZ et al., 2012), while the ACTH and corticosterone concentrations during insulin infusion showed a stronger increase after memantine administration (I et al., 2008). To the best of our knowledge, there are no relevant reports regarding NIM by indomethacin or atorvastatin in the literature. In the present study, we showed the HPA and HPG axes were significantly dysfunctional in APP/PS1 transgenic mice. These disturbances might contribute to the acceleration of Aβ pathogenesis and cognitive function impairments. Our data demonstrated that long-term treatment with a combination of indomethacin and atorvastatin restored HPA and HPG axes function in APP/PS1 transgenic mice.

In conclusion, our data show that indomethacin and atorvastatin treatment restored normal neuroendocrine immune system conditions, and reduced the pathologic changes and cognitive impairments in APP/PS1 transgenic mice. This combination of indomethacin and atorvastatin can thus be a potential therapeutic agent for AD. Based on this study, we did not know whether the doses of indomethacin and atorvastatin that we chose produced maximal effects, and both compounds (indomethacin and atorvastatin) were not tested independently under the same experimental conditions. Future studies will be necessary to further investigate the optimal dose of indomethacin and atorvastatin in the combination, and whether indomethacin plus atorvastatin treatment might be more beneficial than using both drugs independently on improving cognition and regulating Aβ concentration in AD.

Acknowledgements

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