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Oxymatrine exhibits anti-neuroinflammatory effects on $A\beta_{1-42}$-induced primary microglia cells by inhibiting NF-κB and MAPK signaling pathways

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ABSTRACT

Oxymatrine (OMT), isolated from *Sophora flavescens* or *Sophora alopecuroides*, possesses various pharmacological and biological activities, including anti-inflammatory, anti-oxidant, and anti-diabetic properties. Microglia cells, the resident immune cells in the central nervous system (CNS), play a key role in neurodegenerative diseases. In this study, the neuroinflammatory effects of OMT and its mechanisms were investigated by $A\beta_{1-42}$-induced rat brain tissue model and primary microglia cells model. The hematoxylin-eosin (HE) staining and immunohistochemistry results showed that OMT could reduce neuronal damage and inhibit microglia activation in the model tissue. The in vitro experiments revealed that OMT could decrease the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and nitric oxide (NO), and down-regulate the expression of iNOS and COX-2 in a dose-dependent manner. Furthermore, OMT inhibited phosphorylation of JNK, ERK 1/2, P-p38 and NF-κB in $A\beta_{1-42}$-induced microglia cells. In summary, OMT exhibits anti-neuroinflammatory effects and the anti-inflammatory activity of OMT is related to the regulation of MAPK and NF-κB signaling pathways.

1. Introduction

Alzheimer's disease (AD) is a central nervous system degenerative lesion with progressive cognitive dysfunction and memory impairment [1]. AD is one of the common senile diseases [2], which has some pathological features, such as the senile plaque due to Aβ deposition, neuronal loss in the hippocampus and neurofibrillary tangles formed by tau hyperphosphorylation in neurons [3]. AD hypothesis consists of Aβ metabolic abnormalities, tau phosphorylation, inflammatory response, and abnormal free radical metabolism [4,5]. It has been reported that excessive accumulation of Aβ in the brain of AD patients is a main cause of AD [6]. In particular, $A\beta_{1-42}$ is widely and stably present in senile plaques and neurofibrillary tangles [7].

Microglia cells, the resident immune cell of the central nervous system [8], play a key role in the normal brain. When Aβ deposits in a small amount in the brain, microglia cells have phagocytic immunity and inhibit the inflammatory pathway [9]. However, abnormal deposition of Aβ leads to excessive activation of microglia cells [10], which not only accumulate Aβ but also produces TNF-α, IL-1β [11], and increases the levels of iNOS and NO [12], even activate NF-κB and MAPK signaling pathways leads to the increase of inflammation [13]. As a typical inflammatory pathway of neurodegenerative diseases, NF-κB is an inactive complex that is located in the cytoplasm under unstimulated conditions and is in a state of binding to IκB-α [14]. However, once IκB is activated, it is phosphorylated, ubiquitinated, causing the NF-κB to translocate to the nucleus [15]. It reports that MAPK is involved in various cellular processes such as cell growth, proliferation, apoptosis and immune response [16].

OMT is a kind of alkaloid compounds extracted from *Sophora flavescens* [17]. *Sophora flavescens* plays an important role in anti-inflammatory [18], anti-oxidation [19] and antitumor [20]. In addition, a mass of research showed that it can inhibit the expression of TNF-α, IL-1β and IL-6 [21]. OMT has been shown to inhibit some of the inflammatory responses of microglia and delay the development of neurodegenerative diseases [22]. However, the mechanism of OMT against neuroinflammation has not been elucidated. Therefore, we investigated the effects of OMT on pro-inflammatory cytokines and inflammatory pathways.

2. Material and methods

All experiments were conducted in accordance with the regulations

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and general recommendations of Heilongjiang University of Chinese Medicine. Animals were free to obtain water and food, living in the rectangular polypropylene cages at constant temperature (22 ± 1 °C), humidity (50% ± 5%), and maintain 12 h (light)–12 h (dark) cycle conditions.

2.1. Reagents and materials

OMT was obtained from Fang Sheng Biological Development Co. LTD (Baoji, China) with > 98% purity. Dulbecco's modified Eagle's medium (DMEM) was purchased from Corning Cellgro Inc. (Herndon, VA, USA), and the fetal bovine serum (FBS) was purchased from Biological Industries Technologies (Kibbutz Beit Haemek, Israel). $A\beta_{1-42}$ peptide, 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) and TritonX-100 were purchased from Sigma-Aldrich (St Louis, MO, USA). Trypsin-EDTA Solution, Poly-D-lysine (PDL), Ultrasensitive ECL chemiluminescence kit and Hoechst 33258 were obtained from Beyotime Biotechnology Co. Ltd. (Shanghai, China). Penicillin-streptomycin solution was purchased from Hyclone Co. Ltd. (Logan, Utah, USA). Goat serum, CD11b antibody and TRITC-labeled Goat Anti-rabbit IgG were purchased from Santa Cruz (St Louis, MO, USA). All other chemicals and reagents in this work were analytical grade.

2.2. Animals and animal treatments

Adult Sprague-Dawley rats (Provided by the Animal Experiment Centre of Heilongjiang University of Chinese Medicine, SCXK HEI 2008-004) weighing 340–380 g (half male) were used for this experiment.

Fifty rats were randomly divided into five groups, including the Control, Model ($A\beta_{1-42}$), Donepezil ($A\beta_{1-42}$ + 2 mg/kg), OMT 120 mg/kg ($A\beta_{1-42}$ + 120 mg/kg), and OMT 30 mg/kg ($A\beta_{1-42}$ + 30 mg/kg) groups. $A\beta_{1-42}$ peptide was dissolved and diluted in physiological saline to a concentration of 10 μg/μL. Rats were anesthetized by intraperitoneal injection of 5% chloral hydrate. Aβ1–42 (10 μL per rat) was injected into the intracerebroventricular region (AP, −2.8 mm, ML, ± 1.9 mm, DV, −4.2 mm). Rats in the control group were injected with the equivalent volume of physiological saline within 5 min. After 3 days, rats were intragastrically administered Donepezil or OMT for 30 days.

2.3. Tissue preparation

Based on previous water maze experiments [23]. After the water maze experiments, rats were sacrificed for the further experiment purposes. They were anesthetized and perfused with a cold phosphate buffer solution, the brain tissue was carefully removed and placed in 4% pre-cooled paraformaldehyde fixed for 48 h to conduct HE staining and immunohistochemistry experiments, respectively.

2.4. Hematoxylin-eosin (HE) staining

HE staining was used to observe hippocampal neuron morphology. The brain tissue was embedded in paraffin and prepared into 4 μm paraffin sections, which were then dewaxing with xylene and alcohol in sequence. Sections were stained with hematoxylin and eosin and histopathological changes were observed under a 400× optical microscope.

2.5. Immunohistochemistry

The tissue was frozen and made into 4 μm sections for immunohistochemistry experiments. Briefly, sections were quenched with 0.3% H_2O_2 to endogenous peroxidase. It was blocked with 10% goat serum for 1 h. And then the antibody CD11b was added to the sections for 4 °C overnight, incubated with the secondary antibody for 40 min, and developed by 3,3-diaminobenzidine (DAB), observed under a 400× optical microscope.

2.6. Primary microglia cells culture

Sprague-Dawley rats pups (Provided by the Animal Experiment Center of Heilongjiang University of Chinese Medicine, which number is SCXK HEI 2008-015) born within 3 days were sterilized with 75% alcohol and the heads were cut in the ice PBS. The left and right brains were separated as well as the meninges and blood vessels were removed. The brain tissue was cut into pieces and digest with a final concentration of 0.125% trypsin for 15 min at 37 °C. Stop trypsin digestion with the addition of DMEM and then centrifuged at 1000 rpm for 5 min. The medium was transferred into a new flask and continue to culture for 4 h. After the culture bottle was covered with > 80% of the cells, put on a shaker at 200 rpm for 4 h at a constant temperature of 37 °C to obtain the pure microglia cells.

2.7. Immunofluorescence staining

The morphology of microglia cells was identified by immunofluorescence of CD11b. Briefly, microglia cells were plated on sterile coverslips prepared with PDL. While the coverslips were filled with cells, and then the medium was discarded. The cells were fixed with 4% paraformaldehyde for 20 min at 4 °C, 1% Triton X-100 was added for 15 min. 10% goat serum was added to block for 1 h at room temperature, and then the liquid was removed. Primary antibody CD11b (1:200) was added and incubated overnight at 4 °C. A second antibody TRITC-labeled Goat Anti-rabbit IgG (1:500) was added to the coverslip and incubated for 2 h at room temperature. Nuclei were stained with 5 mg/mL Hoechst 33258 and incubated for 10 min at room temperature, the cover slips were sealed with glycerol and observe under a fluorescence microscope.

2.8. Cell viability assay

MTT was used to detect cell viability. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well, the plates were divided into OMT (0, 50, 100, 200, 400, 800, 1000 μg/mL) different concentration groups randomly and incubated for 24 h. The culture supernatants were replaced with media containing 0.5 mg/mL MTT and incubated for 4 h at 37 °C in the dark. Subsequently, the medium was removed carefully and replaced with DMSO 150 μL, the absorbance was measured at 490 nm with a Multi-Detection Microplate Reader (PerkinElmer, VICTOR X3, USA).

2.9. Enzyme-linked immunosorbent assay for TNF-α and IL-1β

The levels of TNF-α and IL-1β were measured by ELISA kits (Nanjing Jiancheng Biological Engineering Institute, China), according to the manufacturer's protocol. Briefly, 24-well plates were divided into Control, Model ($A\beta_{1-42}$ 30 μM), OMT 10 μg/mL ($A\beta_{1-42}$ + 10 μg/mL), OMT 100 μg/mL ($A\beta_{1-42}$ + 100 μg/mL), and OMT 1000 μg/mL ($A\beta_{1-42}$ + 1000 μg/mL) groups randomly. The culture-medium supernatants were collected after 24 h. The cells absorbance was measured at 450 nm by a Multi-Detection Microplate Reader (PerkinElmer, VICTOR X3, USA).

2.10. Nitrite (NO) assay

The nitrite concentration is used to assess the production of NO and was measured by the Griess reaction. In short, according to the manufacturer's protocol (Nanjing Jiancheng Biological Engineering Institute, China), the cells were treated as described above, and the medium was collected and mixed reaction with Griess reagent. The absorbance of the mixture was measured at 525 nm by a multi-detection microplate reader (PerkinElmer, VICTOR X3, USA). The concentration of nitrite was measured by using a standard curve.

2.11. Western blotting

Briefly, microglia cells were seeded on 6-well culture plates. After incubated with OMT (10, 100, 1000 μg/mL) and stimulated with 30 μM $A\beta_{1-42}$ for 24 h, the cells were washed twice with PBS. Cells were lysed by RIPA lysis buffer. After centrifugation at 14,000 rpm for 20 min at 4 °C, the supernatant was separated and the concentration of sample was quantified using a BCA protein assay kit (Beyotime, China). The sample were separated by SDS-PAGE (5% spacer gel and 10% separator gel) and electrically transferred to nitrocellulose membranes. After transfer, the membranes were blocked in 5% skim milk for 2 h at room temperature. Afterwards, the membranes were incubated overnight at 4 °C with primary antibodies against iNOS (1:200), COX-2 (1:200), IκBα (1:1000), P-IκBα (1:1000), P-NF-κB-P65 (1:1000), P-JNK (1:1000), P-ERK 1/2 (1:1000), P-p38 (1:1000) and β-actin (1:1000). After washed three times with TBST for 15 min, membranes were incubated with a second antibody (15000) for 40 min at room temperature. The membranes were visualized using the ECL kits and the Western blot detection reagent, and the bands were scanned by the molecular imager (BIO-RAD, USA).

2.12. Statistical analysis

Experimental results were expressed as the mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Value of $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Histopathology

The purpose of the HE staining experiment was to measure the integrity and order of neurons in the hippocampus. As Fig. 1A–E showed, in the Control and Donepezil groups, there was no obvious neuronal damage, the neuronal cell contours were clear and intact, and the nucleus was round and large, the cells were closely arranged. Compared with the control group, the neurons in the model group damaged, accompanying cells shrink. The chromatin was deepened, and the nuclear shrinkage was smaller. The whole cell arrangement was loose and disordered. The OMT 120 mg/kg treatment group ameliorates the damage of neurons, with less neuron loss, relatively tight arrangement, less cytoplasmic deep staining, and no obvious pyknosis in the nucleus.

3.2. Effect of OMT on microglia immunohistochemistry

It is well known that brownish yellow particles are positive expression of microglia. As shown in Fig. 1F–J, compared with the control group, microglia in the model group stained deeper, more brown-yellow particles, showed irregular morphology. However, compared with the control group, the OMT 120 mg/kg treatment group showed a decrease in brown-yellow particles.

3.3. Immunofluorescence staining of cells

The purity of primary microglia cells was > 95% identified by CD11b immunofluorescence. After observed under the microscope, it was found that the stained cells bodies were fusiform or polygonal. TRITC stained positive neurons show red fluorescence (Fig. 2A), Hoechst 33258 stained nucleus show blue fluorescence ((Fig. 2B).

3.4. Effect of OMT on the viability of primary microglia cells

The effect of OMT at different concentrations on cells viability was examined by MTT method. As shown in Fig. 3, cells incubated with different concentrations of OMT for 24 h and have not detected a significant effect on cells viability compared with the control group. OMT at different concentrations did not have any effect on primary microglia cells, indicating that OMT has no cytotoxic to microglia cells.

3.5. Effect of OMT on TNF-α, IL-1β levels in $A\beta_{1-42}$-induced primary microglia cells

Production of TNF-α and IL-1β play a crucial role in microglial cells activation and neurological inflammation [24]. In this study, the effect of OMT on TNF-α, IL-1β levels were measured by ELISA kits. In Fig. 4A and B, the levels of TNF-α and IL-1β in model group were increased respectively compared with control group. But OMT treatment significantly decreased the levels of TNF-α and IL-1β compared with model group. These findings indicated that OMT could attenuate inflammatory in $A\beta_{1-42}$-induced primary microglia cells in a dose-dependent manner.

3.6. Effect of OMT on NO production and iNOS protein expression in $A\beta_{1-42}$-induced primary microglia cells

As shown in Fig. 4C, the production of NO was significantly

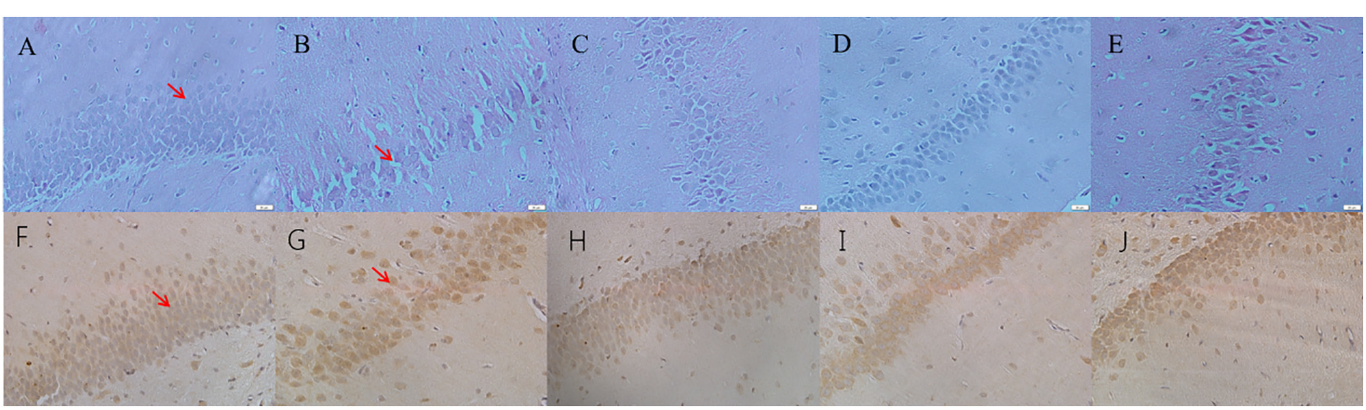

Fig. 1. Effects of OMT on the histopathological changes and immunohistochemistry results in the hippocampus (CA1 region) of $A\beta_{1-42}$-induced Slice of hippocampus A–E are the result of HE staining. (A), Control group (B), Model group ($A\beta_{1-42}$) (C), Donepezil ($A\beta_{1-42}$ + 2 mg/kg) group (D), OMT 120 mg/kg group ($A\beta_{1-42}$ + 120 mg/kg) (E), OMT 30 mg/kg group ($A\beta_{1-42}$ + 30 mg/kg) groups. F–J are the result of immunohistochemistry. (F), Control group (G), Model group ($A\beta_{1-42}$) (H), Donepezil ($A\beta_{1-42}$ + 2 mg/kg) group (I), OMT 120 mg/kg group ($A\beta_{1-42}$ + 120 mg/kg) (J), OMT 30 mg/kg group ($A\beta_{1-42}$ + 30 mg/kg) groups. Scale bar = 20 μm.

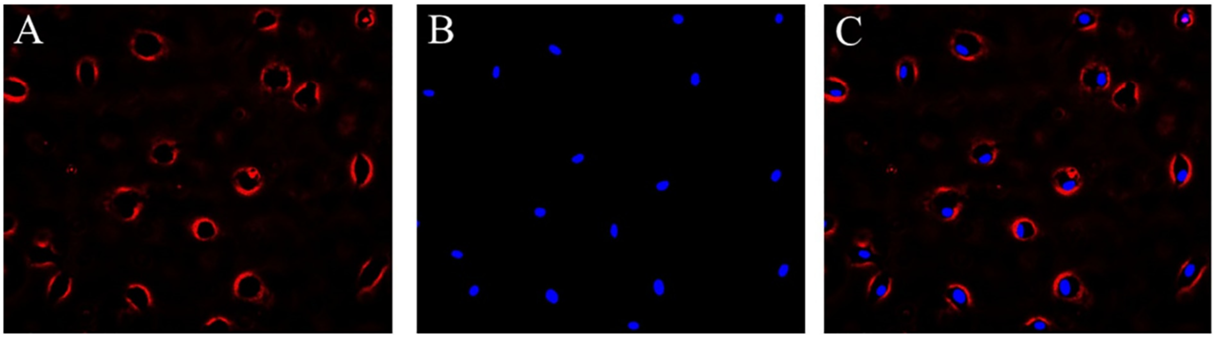

Fig. 2. Immunofluorescence staining of microglial cells (400 × magnification). The purity of primary microglia were identified by CD11b immunofluorescence, CD11b staining showed red fluorescence was microglia morphology (Fig. 2A), Hoechst 33258 nuclear staining was blue (Fig. 2B). Cell morphology and nucleus merge (Fig. 2C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

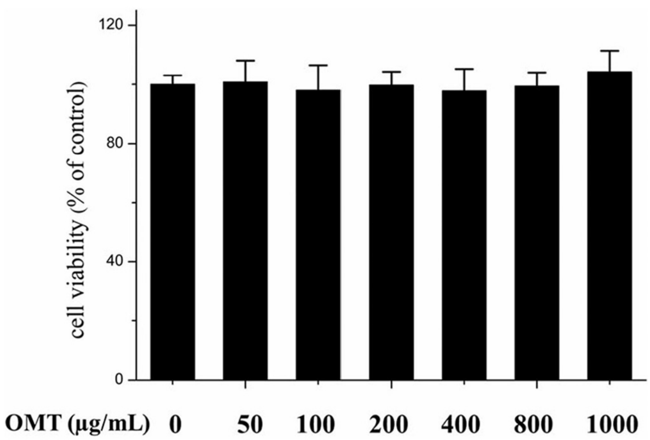

Fig. 3. Effect of OMT on microglia cells viability. (n = 3).

increased in model group compared with control group, but markedly decreased after OMT treatment. Moreover, in Fig. 5A, the expression of iNOS in different concentrations of OMT treatment groups was also decreased in a dose-dependent manner. These data indicate that OMT inhibits the production of iNOS and NO in Aβ_{1-42}-induced primary microglia cells.

3.7. Inhibitory effect of OMT on COX-2 expression in Aβ_{1-42}-induced microglial cells

As COX-2 has been reported to take part in inflammatory responses [25], our study explored the protein expression levels of COX-2 by Western blot method. As shown in Fig. 5B, Aβ_{1-42} treatment group significantly increased COX-2 protein expression in microglial cells. However, the expression of COX-2 was markedly decreased in different concentrations of OMT in a dose-dependent manner.

3.8. Effect of OMT interferes with the phosphorylation of IκBα and P65 in Aβ_{1-42}-induced microglial cells

It is well known that NF-κB requires phosphorylation and proteolytic degradation of IκBα before translocation from the cytoplasm to the nucleus [26]. As shown in Fig. 6, the level of IκBα was significantly lower in model group than control group. After treated with OMT, the expression of IκBα phosphorylation was blocked, and IκBα protein content was obviously increased. In addition, NF-κB-p65 protein content was lower in OMT treatment group than in model group.

3.9. Effect of OMT on JNK, ERK 1/2 and P38 pathway in Aβ_{1-42}-induced microglial cells

It is well known that the MAPK pathway plays an important role in the inflammatory response [27]. In Fig. 7, OMT markedly down-regulated the expression of P-JNK, P-ERK 1/2, P-p38 protein content in MAPK pathway. Therefore, the result indicated that OMT inhibits Aβ_{1-42}-induced microglia cells activation by blocking JNK, ERK 1/2, and P-p38 pathways.

4. Discussion

The damage of hippocampal neuron, a feature of neurodegenerative diseases [28], can be used to assess the extent of the disease. Our in vivo results confirmed that OMT improve learning and memory in rats, and brain tissue section experiments further confirmed this finding. In the

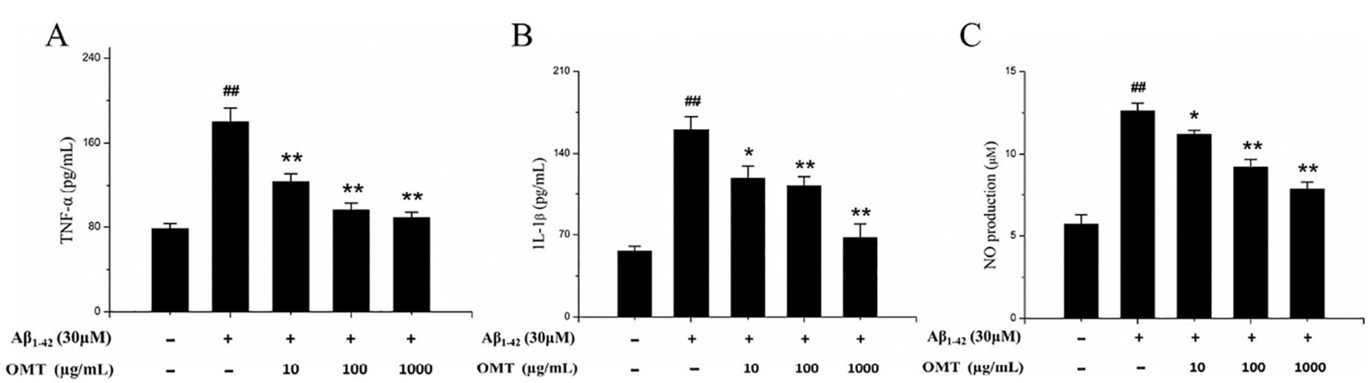

Fig. 4. Effects of OMT on the production of TNF-α, IL-1β and NO in Aβ_{1-42}-induced microglia cells. (A) Production of TNF-α in microglia cells, (n = 3). (B) Production of IL-1β in microglia cells, (n = 3). (C) Production of NO in microglia cells, (n = 3). $^{\#\#}p < 0.01$ vs. Control group, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Aβ_{1-42} group.

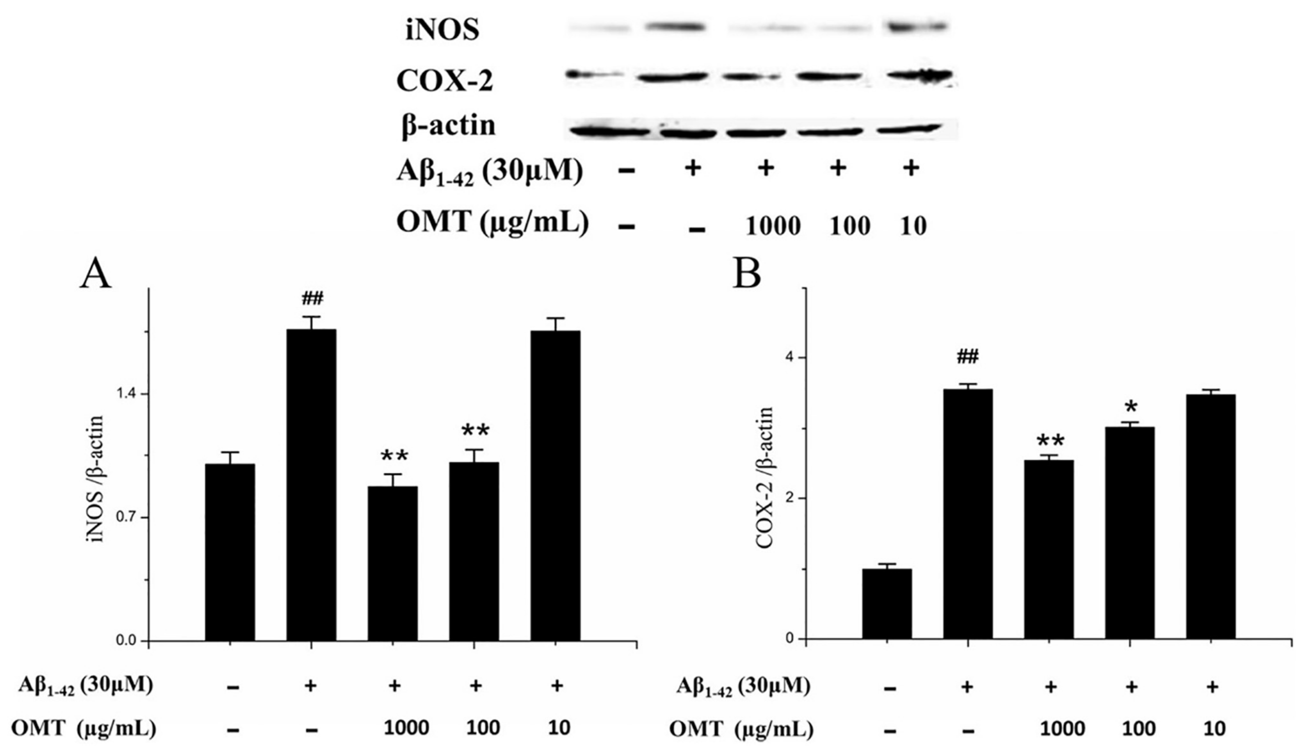

Fig. 5. Effect of OMT on the expression of iNOS and COX-2 in $A\beta_{1-42}$ treated microglia cells. (A) iNOS expression in $A\beta_{1-42}$ treated microglia cells, (B) COX-2 expression in $A\beta_{1-42}$ treated microglia cells, $^{\#\#}p < 0.01$ vs. Control group, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $A\beta_{1-42}$ group.

model group, neuronal cells were severely lost, cells were shrunk and stained, and the nucleus was pyknotic. However, the neurons in the OMT 120 mg/kg group were significantly improved, the nuclei were large and round, the cells were arranged neatly, and no obvious neuron was lost. The results of immunohistochemistry showed that the OMT treatment group had an inhibitory effect on the activation of microglia. Compared with the model group, the microglia in the treatment group showed an inactivated rod shape, and the cells stained lightly, and the neurons could be distinguished. The cells in the model group with round shape were activated microglia. The surrounding cells are seriously damaged, and the expression of dark brown positive microglia was obvious.

In vivo experiments confirmed the expression of microglia in the hippocampus of rats. Therefore, we measured the microglia in vitro. Microglia cells exist in the early development of the brain and are scattered throughout the central nervous system [29]. As a kind of immune cell, it is an important component of the central nervous system [30] and it can response after inflammation stimulate [31]. When a small amount of $A\beta_{1-42}$ stimulation, microglial cells can effectively block the release of pro-inflammatory cytokines [32], and

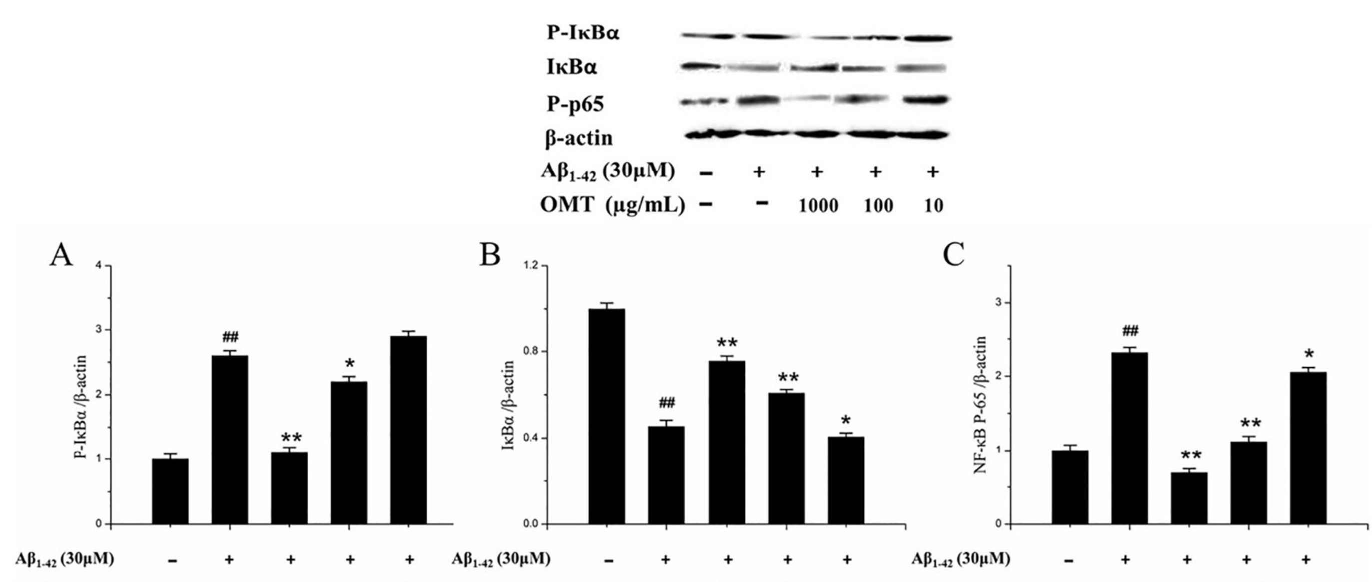

Fig. 6. Effect of OMT on the expression of P-IκBα, IκBα and NF-κB-P65 in $A\beta_{1-42}$ treated microglia cells. (A) P-IκBα expression in $A\beta_{1-42}$ treated microglia cells, (B) IκBα expression in $A\beta_{1-42}$ treated microglia cells, (C) P65 expression in $A\beta_{1-42}$ treated microglia cells. $^{\#\#}p < 0.01$ vs. Control group, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $A\beta_{1-42}$ group.

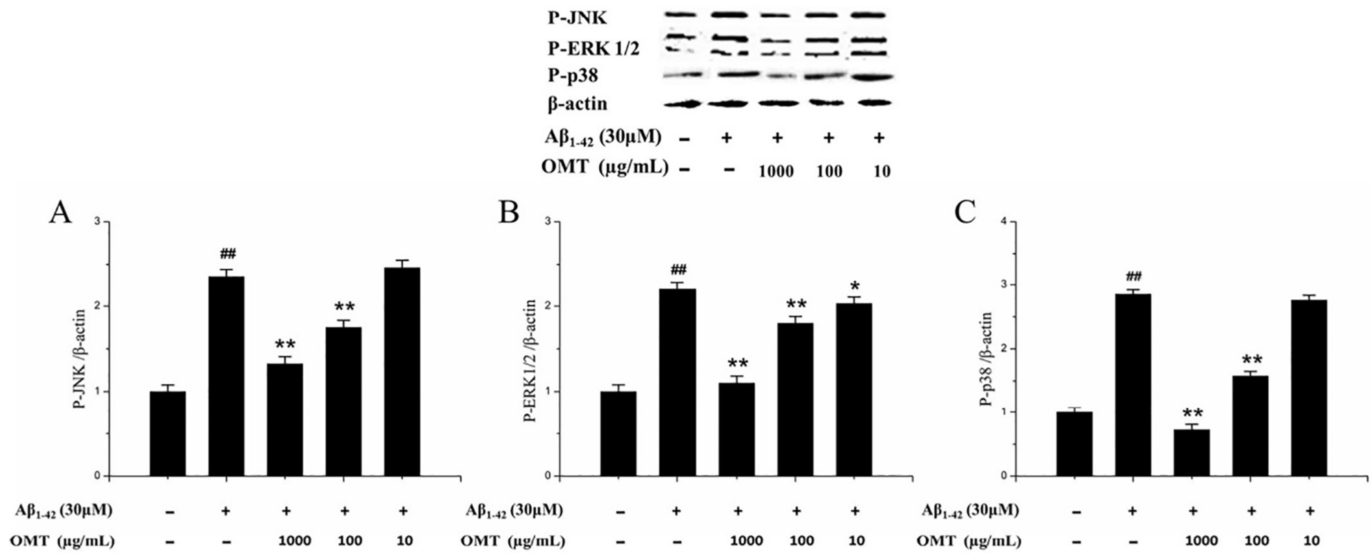

Fig. 7. Effect of OMT on the expression of P-JNK, P-ERK1/2, P-p38 in $A\beta_{1-42}$ treated microglia cells. (A) P-JNK expression in $A\beta_{1-42}$ treated microglia cells, (B) P-ERK1/2 expression in $A\beta_{1-42}$ treated microglia cells, (C) P-p38 expression in $A\beta_{1-42}$ treated microglia cells. $^{\#\#}p < 0.01$ vs. Control group, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $A\beta_{1-42}$ group.

promote tissue repair, release of neurotrophic factor playing an anti-inflammatory effect [33]. However, the excess Aβ cannot be cleared, resulting in microglial cells over-activation lead to the pro-inflammatory cytokines to secrete [34].

In this study, compared with control group, the level of IL-1β and TNF-α in OMT group decreased. This result is consistent with previous research [35]. As the important inflammatory factors, IL-1β and TNF-α can promote the production of neurological inflammation plaques lead to the production of excessive aggregation of Aβ [36]. While the excessive Aβ will activate microglial cells abnormally to produce inflammatory factors, leading to the formation of positive feedback and aggravate AD [37]. These results indicated that OMT has anti-inflammatory effect by suppressing $A\beta_{1-42}$-induced pro-inflammatory cytokines production in microglial cells.

As COX-2 has been reported to take part in inflammatory responses [38], our study explored the protein expression level of COX-2 by Western blot. Many studies have addressed the anti-inflammatory regulation by inhibiting iNOS and COX-2 in cells [39]. Our result showed that the expression of COX-2 significantly decreased in OMT treatment group. Thus, OMT improves inflammatory response caused by microglia activation. In addition, in the three subtypes of NOS, the expression of iNOS can promote the secretion of NO [40]. The increase of NO content will produce neurotoxicity, aggravate neuronal damage [41]. The results of our experiment also demonstrated a positive correlation between NO and iNOS. In this study, we confirmed microglial cells were stimulated by $A\beta_{1-42}$, they activate their immune response mechanism and promote the expression of NO and up-regulate the expression of COX-2. The down-regulation of these indicators slows down the abnormal activation of small gelatin and reduces the damage of nerve cells.

NF-κB and MAPK signaling pathways have important position in neurodegenerative diseases [42]. NF-κB is a nuclear transcription factor that regulates the expression of several inflammation-related factors and plays a major role in the inflammatory response [43]. Under physiological conditions, NF-κB is present in the cytoplasm in an inactive form [44]. Independent of pathological conditions, NF-κB translocate into the nucleus upon stimulation, regulating the gene expression associated with inflammation [45]. In this study, compare with control group, the expression of NF-κB-p65 in Model group significantly increased, but decreased after treated with OMT in microglial cells. It is well known that NF-κB requires phosphorylation and proteolytic degradation of IκBα before translocation from the cytoplasm to the nucleus [46]. Our result showed that IκBα significantly increased in OMT treatment group than model group. Therefore, OMT down-regulated the expression of p65 and up-regulated the IκBα to inhibited NF-κB pathway.

MAPK is an important pathway that mediates inflammation and participates in cytokine control [47]. Many studies have shown that MAPK signaling pathway is involved in LPS-induced microglial cell line inflammatory response [48]. Thus, we suspect that it may be involved in $A\beta_{1-42}$-induced primary microglial cells inflammation. The MAPK family consists of three independent signaling pathways, ERK, JNK, and P38 [49]. The inflammatory response activates the MAPK pathway and experimental results showed that in contrast with the control group, P-ERK1/2, P-JNK, P-p38 in model group was respectively increased. Compared with the model group, the expression of P-ERK1/2, P-JNK, P-p38 in OMT treatment group were markedly decreased. It was shown that OMT inhibits the MAPK pathway in a dose-dependent manner. This result confirmed that OMT indeed inhibit the MAPK signaling pathway and slow down the inflammatory response induced by microglia activation.

5. Conclusion

In summary, our investigation has identified that OMT alleviates neuronal damage, inhibits microglial activation, and improves hippocampal neuronal status in vivo. OMT still down-regulates the expression of iNOS, COX-2, and reduces the production of TNF-α, IL-1β and NO in a dose-dependent manner. Furthermore, OMT also inhibits MAPK and NF-κB inflammatory pathways in vitro. Therefore, OMT might be a promising compound in the development of new therapeutic agents for Alzheimer's disease.

Declaration of Competing Interest

The authors declare they have no conflict of interests.

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