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ORIGINAL ARTICLE

Protective Effects of 1-Methylnicotinamide on $A\beta_{1-42}$-Induced Cognitive Deficits, Neuroinflammation and Apoptosis in Mice

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Abstract

The neurotoxicity of Aβ peptides has been well documented, but effective neuroprotective approaches against Aβ neurotoxicity are unavailable. In the present study, we investigated effects of 1-Methylnicotinamide (MNA), known as a main metabolite of nicotinamide (NA), on the impairment of learning and memory induced by Aβ and the underlying mechanisms. We found that intragastric administration of MNA at 100 or 200 mg/kg for 3 weeks significantly reversed bilateral intrahippocampal injection of $A\beta_{1-42}$-induced cognitive impairments in the Morris water maze (MWM), Y-maze and Novel object recognition tests. Furthermore, MNA suppressed $A\beta_{1-42}$-induced neuroinflammation, characterized by suppressed activation of microglia, decreased the expression of IL-6, TNF-α and nuclear translocation of NF-κB p65, as well as attenuated neuronal apoptosis as indicated by decreased TUNEL-positive cells and ratio of caspase-3 fragment to procaspase-3, and increased ratio of Bcl-2/Bax in the hippocampus. Our results show that MNA may ameliorate $A\beta_{1-42}$-induced cognition deficits, which is involved in inhibition of neuroinflammation and apoptosis mediated by NF-κB signaling, suggesting that MNA could have potential therapeutic value for AD.

Keywords 1-Methylnicotinamide · $A\beta_{1-42}$ · Cognition deficits · Neuroinflammation · Apoptosis

Introduction

Alzheimer's disease (AD) is considered to be a common neurodegenerative disease and characterized by gradual loss of memory, cognitive dysfunction, abnormal behavior and social disorder. Extracellular amyloid-beta (Aβ) protein deposition is one of the major pathogenic mechanisms that act in concert to produce memory dysfunction and decline of cognition. (Frost et al. 2013; Tang et al. 2014a). It has been shown that the deposition of Aβ activates microglia and then induces inflammatory response (Stewart et al. 2010). Activated microglia secretes inflammatory factors, such as TNF-α, IL-6, IL-1β, and a large number of oxidative products, resulting in inflammation of the brain and eventually death of neurons and occurrence of AD (Wang et al. 2015; Qian et al. 2016; Hurley et al. 2018).Numerous studies showed that prolonged infusion of synthetic Aβ into the brain can cause learning and memory deficits in animals (Nitta et al. 1997), including impairment of working memory and place learning in Y-maze, and water maze (Maurice et al. 1996). However, effective neuroprotective approaches against Aβ neurotoxicity are unavailable yet.

1-Methylnicotinamide (MNA) is a main metabolite of nicotinamide (NA) (Przygodzki et al. 2010; Sternak et al. 2015). It is formed in the cells by nicotinamide n-methyltransferase (NNMT) catalyzing the transfer of methyl group of S-adenosylmethionine (SAM) to NA and then metabolized by aldehyde oxidase to 1-methyl-2-pyridone-5-carboxamide (M2PY) or 1-methyl-4-pyridone-5-carboxamide (M4PY) (Chlopicki et al. 2007; Bryniarski et al. 2008; Bar et al. 2017). The evidences were available indicating remarkable pharmacological efficacy of MNA, which was reported that it could be an activator of prostacyclin production and thus may regulate thrombotic as well as inflammatory processes in the cardiovascular system (Chlopicki et al. 2007). Oral administration of

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MNA ameliorated oxidative stress, apoptosis, necrosis, inflammation, and fibrosis in the kidneys of free fatty acid-bound albumin-overloaded mice (Tanaka et al. 2015). Furthermore, MNA possesses hepatoprotective activity, as it can inhibit concanavalin A-induced liver injury through the downregulation of IL-4 and TNF-α signaling (Sternak et al. 2010). Interestingly, it has been reported that MNA may have acquired a normally beneficial role as it inhibited the export of choline from the brain, and therefore boosted acetylcholine levels. This may help developing cognition, delaying the effects of cholinergic degeneration, and eventually providing proper brain growth and development (Williams et al. 2005). MNA also has a role in preventing diabetes-associated brain disorders, and treatment with MNA induced stimulation of serotonin uptake with a normalizing effect on spontaneous neurotransmitter release in rat brain synaptosomes (Kuchmerovska et al. 2010). The studies using cerebellar granule cells have shown that MNA is potentially neuroprotective against the toxicity of glutamate and homocysteine (Slomka et al. 2008a; Slomka et al. 2008b).However, the effects of MNA in AD remain unknown. In this study, we firstly observed the effects of MNA on spatial learning and memory in bilateral intrahippocampal $A\beta_{1-42}$-injected mice. Further, we investigated its possible underlying mechanisms by detecting proinflammatory cytokines and the proteins related to apoptosis in brain.

Materials and Method

Materials

Male Institute of Cancer Research (ICR) mice, approximately 3 months old, weighing 25-30 g, were from Yangzhou University Medical Center (China), all experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Animal Care and Use Committee of China Pharmaceutical University. Mice were fed in an air-conditioned room with a temperature control at about 22 °C, and a 12 h/12 h lighting cycle with free access to water.

$A\beta_{1-42}$ was purchased from Sigma Aldrich (Missouri, USA). MNA for administered intragastrically (i.g.) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The antibodies were purchased from the following companies: anti-cleaved caspase-3, anti-IL-6 and anti-Bcl-2 were from Cell Signaling Technology, Inc. (Massachusetts, USA); anti-Bax and anti-TNF-α were from Santa Cruz Biotechnology, Inc.(Heidelberg, Germany); anti-NF-κB p65 was from Cell Signaling Technology, Inc. (Massachusetts, USA); anti-Iba1 was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); Histone H3, β-actin and secondary antibodies were from Bioworld Technology Co., Ltd. (Minnesota, USA). All other chemicals were commercially available and analytical grade. Before using, $A\beta_{1-42}$ was reconstituted in phosphate-buffered saline (PBS, pH 7.4) at a concentration of 410 pmol/5 μL, and was aggregated by incubation at 37 °C for 7 days (Russo et al. 2012; Lai et al. 2014; Tang et al. 2014b; Zhang et al. 2017; Garcez et al. 2018).

Stereotaxic Intrahippocampal $A\beta_{1-42}$ Infusion and Drug Treatments

Mice were anesthetized using chloral hydrate(350 mg/kg, i.p.) and then fixed on a stereotaxic apparatus (SR-5; Narishige, Tokyo, Japan). The dura was exposed, and a micro syringe attached to a microinjection pump (Longer Pump, China) was inserted into the left and right parietal cortices at a site 2.0 mm caudal to the bregma, 1.5 mm from the midline, and 2.0 mm below the dural surface (Zhang et al. 2016). PBS (0.1 M, pH 7.4, 5 μL) with or without $A\beta_{1-42}$ (410 pmol/mouse) was injected bilaterally into the hippocampus (1 μL/min for all the infusions).The micro pipettes were left in place for 5 min for minimizing reflux of liquid. After surgery, in order to allow the wound to recover mice were fed alone and rested to for 3 days.

After 3 days of recovery, the mice were divided into five groups: Vehicle plus Vehicle (Veh + Veh), $A\beta_{1-42}$ plus Vehicle ($A\beta_{1-42}$ + Veh), $A\beta_{1-42}$ plus MNA 50 mg/kg ($A\beta_{1-42}$ + MNA 50 mg/kg), $A\beta_{1-42}$ plus MNA 100 mg/kg ($A\beta_{1-42}$ + MNA 100 mg/kg), $A\beta_{1-42}$ plus MNA 200 mg/kg ($A\beta_{1-42}$ + MNA 200 mg/kg). The mice were administered intragastrically with MNA (50, 100 and 200 mg/kg, 0.1 ml/10 g of body weight, dissolved in physiological saline) or physiological saline every day for 3 weeks. After supplement of MNA or physiological saline, one part of mice was subjected to behavioral trials and the brain examination was carried out from another part of mice.

Morris Water Maze (MWM) Test

Spatial learning and memory was assessed by the MWM test. Mouse was trained lonely in a round pool with a diameter of 1.2 m; a height of 0.5 m and filled to a depth of 0.3 m with water kept at 25 °C. The round pool was put in a quiet room with visual cues. An escape platform with a diameter of 9 cm was placed in the center of fourth quadrant of the pool. During the five days of training, the platform remained fixed and the position kept unchanged. For each trial, the mouse's face was facing the pool wall and the starting position was pseudo-randomized (Tang et al. 2014a). The test was contained a five-day training period, which was visible and invisible platform training sessions (day 1–2; day 3–5) and a probe trial on day 6. During each trial, every mouse was experienced four quadrants and a 1-h interval between trials. On day 1–2, the platform, bundled with a small flag, was fixed 1 cm below the surface of the water. However, small flag was removed but the

platform remained stationary on day 3, 4 and 5. The baseline differences in vision and motivation can be assessed by visible-platform test. We used hidden-platform version, which evaluates spatial learning, to determine the retention of memory to find the platform. From day one to day five, each test, the total time of 90 s, the mouse ends the experiment after finding the platform and staying for 10 s. Conversely, if the mouse did not find the platform in a specific period of time, it needs to be artificially place on the platform to learn 30 s. On the last day, during the probing test, animals were allowed to swim for 90 s while the platform was taken away. The trend of the mouse to search for platform was measured by the time spent in the target quadrant, where the platform was previously located. The number of target platform location crossings was recorded and processed through the video tracking device and computer equipped analytics management system (Viewer 2 Tracking Software, Ji Liang Instruments, China).

Modified Y-Maze Test

This was performed with slight modification from (Kimura et al. 2010), described elsewhere (Tang et al. 2013). Modified Y-maze test included 2 days. First day is a learning trial, each mouse was placed in one of three compartments and freely moving for 5 min was permitted. After 5 min, the electric shocks (2 Hz, 125 ms, 10 V) in two compartments could be carried out through a stainless steel mesh floor while a shock-free compartments accompanying light sources as clues to enter. Each mouse training 10 times, once the mouse entered the light source of the room and stay for 30 s then the end of training. If the mouse went to the shock-free room at the first instance, it was recorded as a right choice. On the contrary, if not, gently guided the mice to the compartment and allowed to stay for 30 s. The second day (testing trial), the same procedures as the first day, mice were still tested 10 times, but there was no adaptation phase. Manually record the numbers of correct choices and the latency to enter the shock-free compartment on second day.

Novel Object Recognition Test

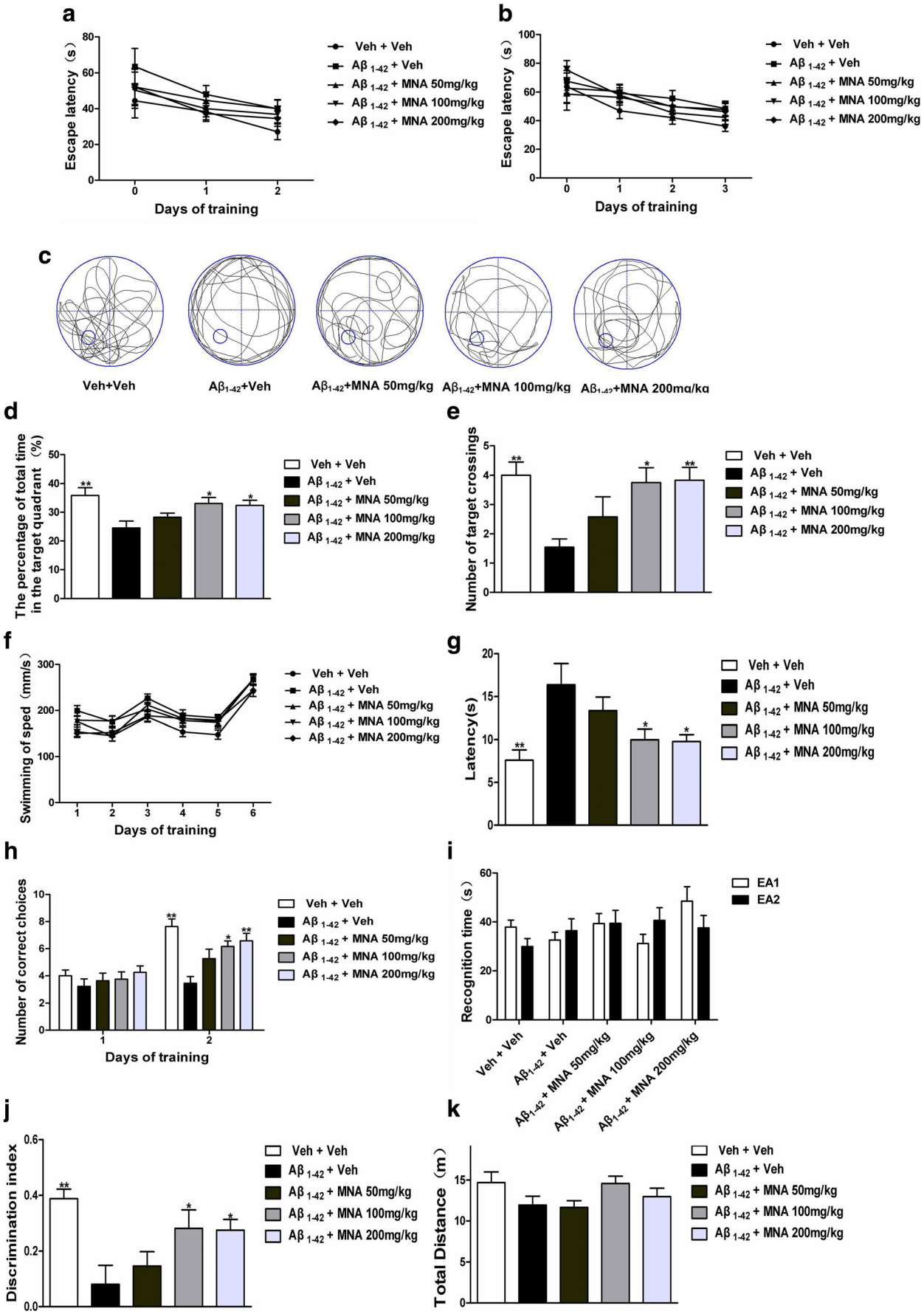
Novel object recognition test (NOR) was applied to detect cognitive function in mice as previously described (Antunes and Biala 2012). The NOR test is based on the spontaneous tendency of mice to exhibit more interactions with a novel rather than a familiar object (Wang et al. 2017). This test consisted of three consecutive days. The first day was a customary stage, animals were allowed to move freely in a rectangular plastic box (50 cm × 50 cm × 40 cm). On the second day, the box contained two identical objects, the exploration time recorded in 5 min. The third day was the testing trial, one of the objects was replaced by a new one, which was a different color and shape object. During the three days, anything that explored behavior of the mouse within 5 min (less than 2 cm away from the object and touched the object with the mouth, nose, or paw) was recorded by the computer. After each mouse ended the test, 70% ethanol was used to eliminate the urine and excrement so as not to affect the next mouse test. The probing time for the same objects A and B during the familiar period was defined as EA1 and EA2; in the testing trial, the investigative time for original object A and new object B was EA and EB. The discrimination index was determined by performing the following calculation: (EB − EA)/(EB + EA). All data were used to assess mice learning and memory behavior of old and new objects.

Open Field Test

Open field test (OFT) was a typical experiment performed to assess spontaneous motor activity and exploration behavior (Huang et al. 2016). The OFT is conducted in a quiet environment. The test arena, a rectangular plastic box (50 cm × 50 cm × 40 cm) was divided into 144 squares. With face toward the box wall, the mouse was gently put in any corner square of the arena. The mouse's exploration behavior in the box was allowed and the total distance of the mice within 5 min was recorded. After each mouse finished the test, the device was washed with 70% ethanol to eliminate the odor.

Tissue Preparation and Immunohistochemistry (IHC)

For analyzing hippocampal microglia activation, mice were anesthetized and transcardially perfused with 0.1 M PBS, followed by 4% paraformaldehyde fixation. Then the brains were taken out, and submerged in 4% paraformaldehyde at 4 °C for 24 h and cryoprotected in a 30% sucrose solution for an additional 24 h. Finally the brains were embedded into optimal cutting temperature compound (Tissue-Tek, Torrance, CA) and cryosectioned (30 μm). The Iba1 immunohistochemistry protocol was conformed to the streptavidinbiotin complex (SABC) IHC kit (Boster Biotechnology Co., Ltd., Wuhan, China). Sections were washed with PBS (3 × 5 min), and then heated on a water bath with 0.3% Triton X-100 for 4 h at 60 °C then treated with 3% H_2O_2 at room temperature for 30 min. After washing with PBS (3 × 5 min), sections were blocked with 5% BSA for 30 min and incubated in anti-Iba1 (1:1000) primary antibody diluted in 5% BSA overnight at 4 °C. Sections were washed with PBS (3 × 5 min) and incubated with biotinylated mouse anti-rabbit IgG (40 min, 37 °C). After washing in PBS (3 × 5 min, 37 °C), slices were incubated with streptavidin-biotin complex (20 min, 37 °C) and washed with PBS again (4 × 5 min). Diaminobenzidine (DAB) was utilized as the final chromogen. After that, 70% ethanol for 5 min, 95% ethanol for 5 min, and 100% ethanol for 2 × 5 min and xylene for 2 × 5 min were performed for gradient dehydration. Sections were then covered with dibutyl phathalate xylene


a
Escape latency (s)
Days of training
Veh + Veh
Aβ 1-42 + Veh
Aβ 1-42 + MNA 50mg/kg
Aβ 1-42 + MNA 100mg/kg
Aβ 1-42 + MNA 200mg/kg
b
Escape latency (s)
Days of training
c
Veh+Veh
Aβ1-42+Veh
Aβ1-42+MNA 50mg/kg
Aβ1-42+MNA 100mg/kg
Aβ1-42+MNA 200mg/kg
d
The percentage of total time in the target quadrant (%)
e
Number of target crossings
f
Swimming of sped (mm/s)
Days of training
g
Latency(s)
h
Number of correct choices
Days of training
i
Recognition time (s)
EA1
EA2
Veh + Veh
Aβ1-42 + Veh
Aβ1-42 + MNA 50mg/kg
Aβ1-42 + MNA 100mg/kg
Aβ1-42 + MNA 200mg/kg
j
Discrimination index
k
Total Distance (m)

◀ **Fig. 1** MNA improves $A\beta_{1-42}$ -induced cognitive deficits in mice. Day 0 indicates performance on the first trail and subsequent points represent an average of all daily trails in the MWW task. **a** During the two days visible platform test, there were no differences in the escape latency among all groups. **b** Escape latency was changed on hidden platform during the 3-day acquisition trials. **c** A representative swimming path and **d** the percentage of total time spent in the target quadrant during the probe trial day. **e** The number of platform crossings during the probe trial test on day 6. **f** Average swimming speed among all groups in the 6 days tests. **g** The latency to enter the shock-free part onday 2 and **h** the number of correct choices during day 1 and day 2 was measured in Y-maze test. **i** The time to explore the same objects A and B during the familiar period was defined as EA1 and EA2. **j** The discrimination index was determined by performing the following calculation: (EB − EA)/ (EB + EA). **k** The total distance in the OFT was shown. Values shown are expressed as mean ± SEM; n = 12. $*p < 0.05$, $**p < 0.01$ versus $A\beta_{1-42}$ + Veh

(DPX) mounting solution and cover glass. Finally, the sections were observed with a light microscope (Leica Microsystems AG, Germany) at a fixed magnification (× 200). The photomicrographs were quantified using Image-Pro Plus software. The number of microglia in the hippocampus was measured, followed by the microglial-positive area to generate the ratio of microglial staining to hippocampal area (% area occupied). The mean values from 4 sections of each mouse were used for statistical analysis.

TUNEL Staining

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) staining can identify nuclei with fragmented DNA (a characteristic of apoptotic cells) by using the in situ cell death detection kit (Roche, Germany). The sections were fixed in 4% PFA (20 min, 37 °C), then washed in PBS (pH 7.4) (30 min, 37 °C). The sections were incubated in permeabilisation solution (0.1% Triton X-100, 0.1% sodium citrate, freshly prepared) for 2 min on ice. TUNEL mixture was added onto brain sections and incubated in a humidified chamber (60 min, 37 °C) and washed in PBS (pH 7.4) (2 × 5 min, 37 °C). Sections were incubated for 10 min in a dark place for DAPI staining. The cells were observed through a fluorescence microscope under a fixed magnification (× 200) (Leica Microsystems AG, Germany). DAPI nuclear staining was used to determine the total number of cells in a given area. $TUNEL^+$ cells were identified by the co-localization of both the TUNEL signal and DAPI. The apoptotic bodies were expressed as a percentage of the total number of cells examined. The mean values from 4 sections of each mouse were used for statistical analysis.

Western Blot (WB) Analysis

Mouse hippocampus were chopped into small pieces and homogenized in ice-cold RIPA (50 mM Tris–HCl (pH 7.4), 150 nM NaCl, 1 mM PMSF, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS). The dissolved proteins were collected from the supernatant after centrifugation at 4 °C, 12000 rpm for 15 min. Protein concentration was determined by a BCA protein assay kit according to the manual (Beyotime Biotechnology, Jiangsu, China) and then assessed for expression of pro- or cleaved caspase-3, Bax, Bcl-2, IL-6 and TNF-α proteins. Protein extracts were separated by a SDS-polyacrylamide gel electrophoresis and then transferred onto a PVDF membrane. The membrane was blocked with 5% BSA in Tris buffer saline and then incubated at 4 °C overnight with respective primary antibodies for anti-pro or cleaved caspase-3 antibody (1:1000), anti-Bcl-2 antibody (1:1000), anti-Bax antibody (1:500),anti-IL-6 antibody (1:1000), anti-TNF-α antibody (1:500), or β-actin (1:5000). After washing with tris buffered saline–tween 20 (TBST), the membranes were incubated with a horseradish peroxidase conjugated secondary antibody (1:10000, Bioworld Technology) for 2 h at room temperature. The antibody-reactive bands were visualized by using enhanced chemiluminescence detection reagents and a gel imaging system (Tanon Science & Technology Co., Ltd., China).

Nuclear extract was performed using nucleoprotein extraction kit (Sangon Biotech, China). At the start, mouse hippocampus was minced and homogenized in ice-cold hypotonic buffer, which including 0.5% phosphatase inhibitor, 1% PMSF and 0.1% DL-Dithiothreitol (DTT), then centrifuged at 4 °C, 3000 rpm for 5 min. The supernatant was discarded and the precipitate was washed in hypotonic buffer and centrifuged 5000 rpm for 5 min at 4 °C. At the end, the precipitate was put into 0.2 mL lysis buffer (containing 0.1% DTT, 0.5% phosphatase inhibitor and 1% PMSF), chilled for 20 min and centrifuged at 15000 rpm for 10 min at 4 °C. The supernatant nuclear protein extract was subjected to Western blot for assay of NF-κB p65 (1:1000) and Histone H3 (1:500) was used as a loading control.

Statistical Analyses

The data are shown as mean ± SEM. Group differences in the MWM test escape latency were analyzed using a repeated measure ANOVA with 'days' as the 'within-subject factor' and 'group' as the between-subject factor. All other data were analyzed by a one-way ANOVA followed by a Dunnett's post hoc analysis for multiple comparisons. All analyses were carried out using SPSS v20.0. The level of statistical significance was $p < 0.05$.

Results

MNA Improves $A\beta_{1-42}$ -Induced Cognitive Deficits in Mice

To determine the effects of MNA on $A\beta_{1-42}$ -induced cognitive deficits in mice, the different behavior tests were carried

out. Firstly, the performance of mice in a non-spatial visible-platform variant of the MWM to test for baseline differences in vision and motivation among treatment groups were assessed. Mice in each group exhibited similar escape latency, suggesting there was no influence on vision or basal motivation among all groups. (4 trials/day for 2 days; effect of day, $F_{(4,517)} = 7.531$, $p = 0.0007$; effect of group, $F_{(4,517)} = 1.651$, $p = 0.1642$; effect of group-by-day interaction, $F_{(4,517)} = 0.1725$, $p = 0.9942$; Fig. 1a). We then tested the mice in the spatial hidden-platform variant, and the data showed that $A\beta_{1–42}$ increased escape latencies compared to the control, these were reversed by MNA (100 or 200 mg/kg) (4 trials/day for 3 days; effect of day, $F_{(4,749)} = 9.658$, $p < 0.0001$; effect of group, $F_{(4, 749)} = 1.377$, $p = 0.2430$; effect of group-by-day interaction, $F_{(4, 749)} = 0.4241$, $p = 0.9529$; Fig. 1b). In the probe trial (1 trial/mouse for day 6), all the mice showed preference for the target quadrant, with the exception of the mice in the $A\beta_{1–42}$ plus vehicle group, which displayed significant decreases in the time in the target quadrant and the number of platform location crossings compared to control group ($p < 0.01$). However, mice in the $A\beta_{1–42}$ plus MNA (100 or 200 mg/kg) groups showed significant increases in both indices compared to $A\beta_{1–42}$ plus vehicle ($p < 0.05$, $F_{(4, 53)} = 4.310$, $p = 0.0043$ for the percentage of total time spent in the target quadrant and $p < 0.05$ or $p < 0.01$ for the number of target crossing, respectively; $F_{(4, 53)} = 4.375$, $p = 0.0039$; Fig. 1d, e). But MNA at the dosage of 50 mg/kg didn't produce significant effects ($p > 0.05$; Fig. 1d, e). In addition, all the mice had similar swim speeds in the trials (Fig. 1f).

To confirm the results observed in the MWM test, we also carried out the Y-maze test. The results showed that MNA (100 or 200 mg/kg) dramatically decreased the latency to enter the shock-free part ($p < 0.05$) and increased the number of correct choices($p < 0.05$ or $p < 0.01$, respectively) compared to $A\beta_{1–42}$ alone ($F_{(4, 50)} = 5.077$, $p = 0.0016$ for the latency to enter the shock-free compartment; $F_{(4,50)} = 0.6816$, $p = 0.6341$ for the number of correct choices; Fig. 1g, h).

Furthermore, the mice were submitted to the NOR for detecting non-spatial learning and memory. Our data indicated that there was no observable difference in the time to explore same objects in all groups of mice ($p > 0.05$; Fig. 1i). During the test period, $A\beta_{1–42}$ caused a significant decline in the discrimination index compared with the control group ($p < 0.01$; Fig.1j), suggesting that $A\beta_{1–42}$-treated mice have impaired object recognition memory. MNA (100 or 200 mg/kg) treatment showed a significant increase in discrimination index compared with the $A\beta_{1–42}$ + Veh group ($p < 0.05$; $F_{(4, 50)} = 4.884$, $p = 0.0021$; Fig. 1j).

In addition, to determine whether general locomotor activity interferes with cognitive behavior test, we conducted the OFT. The results showed that there was no significant difference among the groups in the total distance traveled in the open field test ($F_{(4, 50)} = 1.915$; $p = 0.1216$; Fig. 1k). These behavior data indicate that MNA improves $A\beta_{1–42}$ -induced cognitive deficits in mice.

MNA Prevents Hippocampal Apoptotic Responses in $A\beta_{1–42}$-Induced Mice

Apoptosis in neuronal cells plays a critical role in the pathogenesis of AD (Song et al. 2018). We presented apoptosis in the hippocampus by TUNEL staining to evaluate that MNA play an anti-apoptotic role in $A\beta_{1–42}$-injected neurotoxicity. $A\beta_{1–42}$ treatment caused a significant increase in TUNEL-positive cells in the hippocampus ($p < 0.01$), and MNA (100 or 200 mg/kg) treatment decreased the number of apoptotic cells compared to the $A\beta_{1–42}$ + Veh group ($p < 0.05$; $F_{(4, 70)} = 7.898$, $p = 0.0012$, Fig. 2a, b).We also detected apoptosis-related proteins by the WB assay. The results showed that the ratio of caspase-3 fragment to procaspase-3 was significantly increased and the ratio of Bcl-2/Bax was significantly decreased in the hippocampus of $A\beta_{1–42}$-injected mice ($p < 0.01$, Fig. 2c, d, e and f). MNA (100 or 200 mg/kg) treatment attenuated these changes ($p < 0.05$, $F_{(4, 15)} = 4.966$, $p = 0.0094$ for caspase-3 activation; $p < 0.05$, $F_{(4, 15)} = 6.696$, $p = 0.0027$ for Bcl-2/Bax, Fig. 2c, d, e and f). The results showed that MNA inhibited $A\beta_{1–42}$-induced hippocampal apoptotic responses in mice.

MNA Decreases the Production of Proinflammatory Cytokines Induced by $A\beta_{1–42}$ in Mice

Inflammatory mediators play an important role in cognitive dysfunction (Echeverria et al. 2016; Pfau and Russo 2016; Kempuraj et al. 2017). To determine the effect of MNA on $A\beta_{1–42}$-induced proinflammatory cytokine production in the hippocampus, we examined pro-inflammatory cytokines such as TNF-α and IL-6 by the WB analysis. As shown in Fig. 3, in $A\beta_{1–42}$-treated mice, the increased levels of TNF-α, and IL-6 were observed in the hippocampus ($p < 0.01$), while MNA (100 or 200 mg/kg) treatment significantly reduced their levels in this region ($p < 0.05$ or $p < 0.01$; $F_{(4, 15)} = 5.624$; $p = 0.0057$ for TNF-α; $p < 0.05$;($F_{(4, 15)} = 5.127$; $p = 0.0083$ for IL-6; Fig. 3a, b, c and d). These findings suggest that MNA could protect against $A\beta_{1–42}$-induced neuroinflammation.

MNA Suppresses $A\beta_{1–42}$-Induced Microglia Activation in the Hippocampus

Microglia, the immune cells of the CNS, become chronically activated by amyloid-β peptides (Lowry and Klegeris 2018), following morphological changes and an increase in the number of activated microglia.(Lin et al. 2017). The activated microglia exhibit increased expression of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and IL-6 that can cause neuronal damage (Wyss-Coray 2006). We

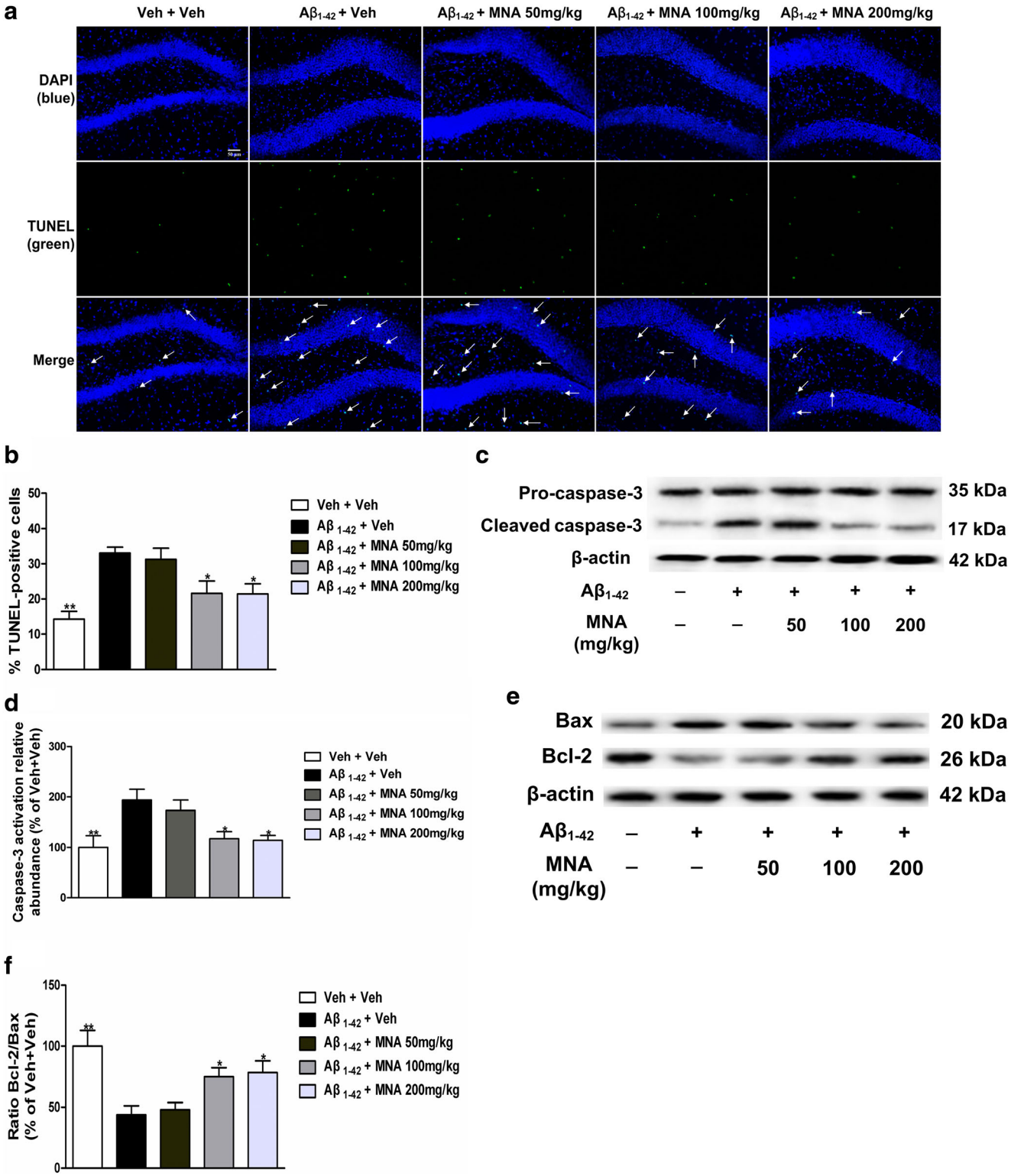


Fig. 2 MNA prevents hippocampal apoptotic responses in the $A\beta_{1-42}$-induced mice. **a** Representative photographs of apoptotic cells shown by white arrows. **b** Results of percentage (%) of apoptosis. **c** Representative bands of pro- or cleaved caspase-3 and **e** Bcl-2 and Bax subjected to the WB assay were shown. **d** Caspase-3 activation was shown as the ratio of caspase-3 to pro-caspase-3 and **f** Bcl-2 and Bax was expressed as the ratio (in percentage) of vehicle plus vehicle group. Values shown are expressed as mean ± SEM; $n = 4$. $^{*}p < 0.05$, $^{**}p < 0.01$ versus $A\beta_{1-42}$ + Veh. Scale bar, 50 μm

detected Iba1, a marker of microglial activation, using IHC assay to determine whether the anti-inflammatory effect of MNA is mediated through suppression of microglia activation. As shown in Fig. 4, $A\beta_{1-42}$ treatment increased the

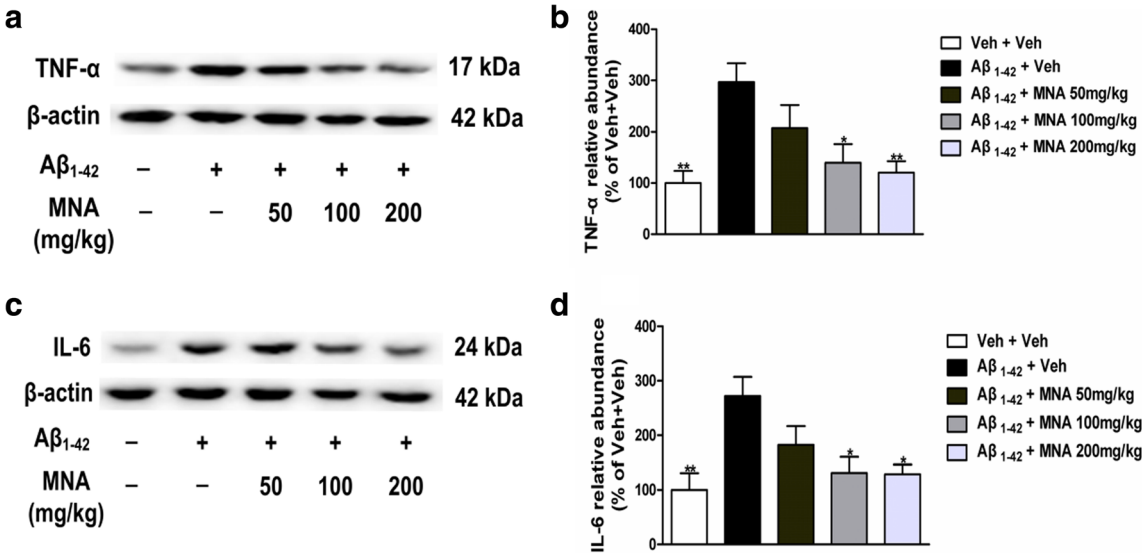


Fig. 3 MNA decreases the production of proinflammatory cytokines induced by $A\beta_{1-42}$ in mice. **a**, **b** TNF-α and **c**, **d** IL-6 and β-actin as loading control were examined by the WB by respective antibodies, and quantification of TNF-α and IL-1β were presented as the ratio (in percentage) of Veh + Veh group. Data shown are expressed as mean ± SEM; n = 4. $^{*}p < 0.05$, $^{**}p < 0.01$ versus $A\beta_{1-42}$ + Veh

number of Iba1-positive cells in the hippocampus ($p < 0.01$), and MNA (100 or 200 mg/kg) treatment efficiently suppressed the activated microglia in the hippocampus ($p < 0.05$ or $p < 0.01$, respectively; $F_{(4, 56)} = 8.815$; $p = 0.0007$; Fig. 4a, b).

MNA Suppresses $A\beta_{1-42}$-Activated NF-κB Signaling in the Hippocampus

Many of the target genes regulated by NF-κB p65 are associated with immune-inflammatory responses and apoptosis (Takeda and Akira 2004; Tang et al. 2018). To investigate whether MNA is associated with $A\beta_{1-42}$-activated NF-κB p65 signaling, the level of nuclear NF-κB p65 in the hippocampus was detected by the WB analysis. Our data showed that $A\beta_{1-42}$ activated NF-κB pathway, as shown by p65 increase in the nucleus. Interestingly, MNA (100 or 200 mg/kg) treatment was effective in blocking $A\beta_{1-42}$-activated NF-κB signaling in the hippocampus ($p < 0.05$; $F_{(4, 15)} = 4.382$; $p = 0.0152$; Fig. 5a, b). This data suggest that inhibition of the nuclear translocation of NF-κB may be a critical mechanism by which MNA mediates its protective effect against $A\beta_{1-42}$-induced neuroinflammation and apoptosis.

Discussion

The aggregation or deposition of Aβ is the key event in AD pathology, which causes cognitive deficits, neuronal apoptosis and neuroinflammation (Pujadas et al. 2014; Piirainen et al. 2017). Here, we demonstrated that a single micro-infusion of $A\beta_{1-42}$ into the hippocampus produced cognition deficits accompanied with neuroininflammation as indicated by activated microglia and increased TNF-α and IL-6, and apoptotic responses as evidenced by increased TUNEL-positive cells, activation of caspase-3, and decreased ratio of Bcl-2/Bax, as well as increased nuclear translocation of NF-κB p65 in the hippocampus. Treatment with MNA (100 or 200 mg/kg) markedly attenuated these effects induced by $A\beta_{1-42}$. These results suggest that MNA improves $A\beta_{1-42}$-induced cognitive deficit, which is at least partially contributed to by inhibiting neuroinflammation and apoptosis mediated by NF-κB signaling.

It was reported that NA, the precursor of MNA, plays a critical physiological role in cellular metabolism (Lin and Guarente 2003; Magni et al. 2004). It has ability to govern cellular integrity and cellular inflammation through modulating microglial activation (Li et al. 2006) and inhibits generation of pro-inflammatory cytokines, such as IL-1β, IL-6, IL-8 and TNF-α (Ungerstedt et al. 2003). NA can also prevent apoptotic injury by impacting cellular pathways that involve DNA fragmentation and membrane phosphatidylserine exposure (DiPalma and Thayer 1991; Chong et al. 2002, 2004).The treatment of transgenic AD mice with NA, improved Aβ and Tau pathologies and ameliorated learning and memory deficits (Liu et al. 2013; Kerr et al. 2017). In the recent years, the role of MNA, as a main metabolite of NA, was emphasized in the CNS. There are some epidemiological data indirectly suggesting that MNA may be neurotoxic and might play a role in the pathogenesis of Parkinson's disease (Williams et al. 2005). Some experimental studies showed that MNA have beneficial effects in hypoxic-ischemic brain damage (Dragun et al. 2008) and the diabetes-associated brain disorders (Kuchmerovska et al. 2010). In the present study, we observed the effects of MNA at the dose of 50, 100 and 200 mg/kg on $A\beta_{1-42}$-induced memory impairment in mice, and find that MNA at 50 mg/kg shows ameliorative effect but this effect didn't reach statistical

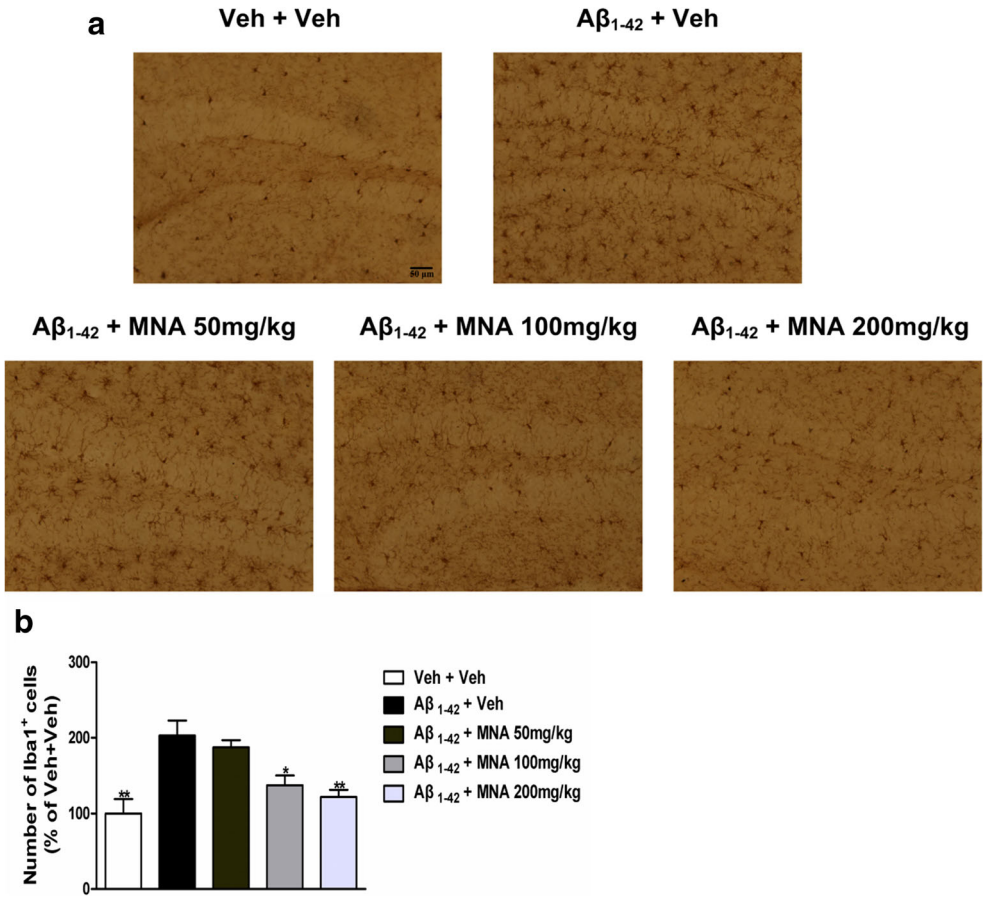


Fig. 4 MNA suppresses $A\beta_{1–42}$-induced microglia activation in the hippocampus. **a** Representative IHC images of microglia activation marker Iba-1. **b** The number of Iba1 antibody-stained microglia was normalized in the corresponding same area, as the ratio (in percentage) of the Veh + Veh are shown. Data shown are expressed as mean ± SEM; n = 4. $^{*}p < 0.05$, $^{**}p < 0.01$ versus $A\beta_{1–42}$ + Veh. Scale bar, 50 μm

significance, whereas MNA at 100 mg/kg produces similar potentially neuroprotective effects to that at 200 mg/kg in the mice, which indicates 100 mg/kg is probably the dosage of maximum effects. These indicate that its dose-response relationship is not significant. Although MNA as a charged organic cation could cross the blood brain barrier (BBB) (Erb et al. 1999), we speculate that its transport across the BBB might be in the transporter-dependent manner. This implies that MNA at 100 mg/kg might produce the maximum concentration in the brain because of the limitation of transport at the BBB. The exact mechanism remains to be further investigated.

Growing evidence suggests that neuroinflammation characterized by excessive activation of microglia and the production of a large number of free radicals and inflammatory factors plays a crucial role in the pathophysiology of AD (Nasoohi et al. 2018). Microglia, as key cellular elements of the acute neuroinflammatory response, is the main source of pro-inflammatory mediators in the brain (Rahimifard et al. 2017). Activation of microglia induces the production of inflammatory cytokines, including IL-6 and TNF-α, which can damage neurons and cause neuronal apoptosis (Rubio-Perez and Morillas-Ruiz 2012; Ding et al. 2018). Hippocampal

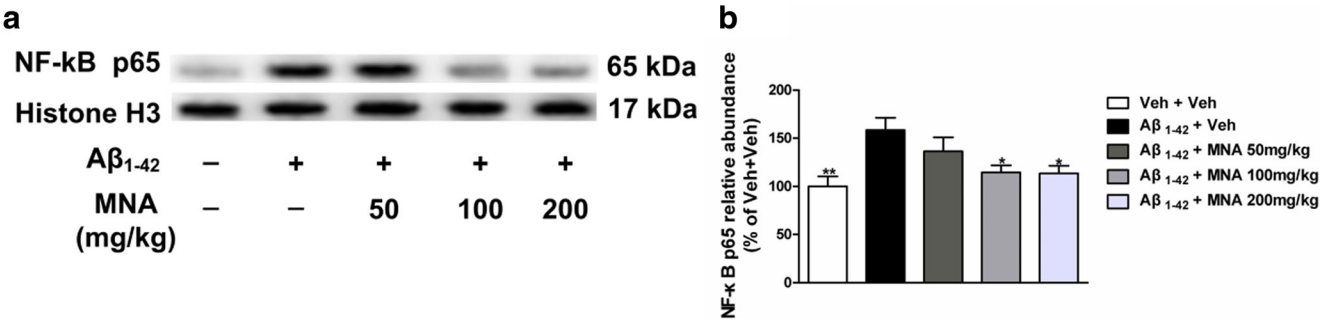


Fig. 5 MNA suppresses $A\beta_{1–42}$-activated NF-κB signaling in the hippocampus. (**a**, **b**) The extraction process of nuclear protein in mouse hippocampus was described above, and nuclear NF-κB p65 and histone H3 as loading control were examined by WB using respective antibodies. Data shown are expressed as mean ± SEM; n = 4. $^{*}p < 0.05$, $^{**}p < 0.01$ versus $A\beta_{1–42}$ + Veh

apoptosis is account for the neuronal loss and the concomitant emergence of cognitive impairments in AD (Lin et al. 2016; Pandey et al. 2017). Bax and Bcl-2, belonging to pro- or anti-apoptotic gene families, mediate apoptosis mainly via regulating the release of cytochrome C and the mitochondrial pathway, as well as by mediating caspases (Ruiz-Vela et al. 2005; Jiao et al. 2012). And caspase-3, as the executor of apoptosis, is a key protease in mammalian cell apoptosis. In this study, as we expected, $A\beta_{1-42}$ treatment triggered microglia activation indicated as the increased number of Iba1-positive cells, subsequently, resulting in the increased release of TNF-α and IL-6 in the hippocampus. Moreover, $A\beta_{1-42}$ treatment enhances apoptosis as evidenced by increased TUNEL-positive cells, activation of caspase-3, and decreases ratio of Bcl-2/Bax in the hippocampus. However, MNA improved $A\beta_{1-42}$-induced cognition deficits, also suppressed the $A\beta_{1-42}$-induced inflammatory and apoptotic responses in the hippocampus.

NF-κB is an important transcription factor that involved in proinflammatory gene activation as well as other genes regulations. It was reported that inhibition of NF-κB transcriptional activity in the microglial nucleus could suppress proinflammatory cytokine production, such as TNF-α and IL-6, and etc. (Ye and Johnson 2001; Sethi et al. 2008). Furthermore, NF-κB signaling enhances apoptosis (Khan et al. 2009; Tusi et al. 2010) and inhibition of NF-κB not only protracts inflammatory responses but also prevents apoptosis (Lawrence et al. 2001). It has been reported that administration of Aβ in excess physiological concentrations can activate NF-κB p65 signaling pathway (Liu et al. 2015). Recent studies have shown that MNA can inhibit activation of the p38 MAPK pathway to decrease apoptosis in cells (Xie et al. 2016). The activation and phosphorylation of p38 MAPK pathways are required for NF-κB p65 nuclear translocation (Je et al. 2004). Our data showed that $A\beta_{1-42}$ increases the nucleus NF-κB p65, which indicated activation of NF-κB signaling. Importantly, MNA treatment significantly reduced the hippocampal nucleus NF-κB p65 level, suggesting decrease of NF-κB signaling. Therefore, the anti-neuroinflammatory and anti-apoptotic effects of MNA are associated with NF-κB signaling in the $A\beta_{1-42}$-induced mice.

Conclusion

In summary, in the present study, we provided promising demonstration for the potentially neuroprotective effects of MNA against neurotoxic of Aβ. These beneficial effects include the improvement of memory deficits and the ameliorations of neuroinflammatory and apoptotic responses. These results suggest that the application of MNA may be a novel and promising strategy to treat or prevent the learning and memory deficits in neurodegenerative diseases such as AD.

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Compliance with Ethical Standards The National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised, 1996) was used for the experiments and the procedures were approved by the Animal Care and Use Committee, China Pharmaceutical University.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Antunes M, Biala G (2012) The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 13: 93–110

Bar A, Olkowicz M, Tyrankiewicz U, Kus E, Jasinski K, Smolenski RT, Skorka T, Chlopicki S (2017) Functional and biochemical endothelial profiling in vivo in a murine model of endothelial dysfunction; comparison of effects of 1-Methylnicotinamide and angiotensin-converting enzyme inhibitor. Front Pharmacol 8:183

Bryniarski K, Biedron R, Jakubowski A, Chlopicki S, Marcinkiewicz J (2008) Anti-inflammatory effect of 1-methylnicotinamide in contact hypersensitivity to oxazolone in mice; involvement of prostacyclin. Eur J Pharmacol 578:332–338

Chlopicki S, Swies J, Mogielnicki A, Buczko W, Bartus M, Lomnicka M, Adamus J, Gebicki J (2007) 1-Methylnicotinamide (MNA), a primary metabolite of nicotinamide, exerts anti-thrombotic activity mediated by a cyclooxygenase-2/prostacyclin pathway. Br J Pharmacol 152:230–239

Chong ZZ, Lin SH, Maiese K (2002) Nicotinamide modulates mitochondrial membrane potential and cysteine protease activity during cerebral vascular endothelial cell injury. J Vasc Res 39:131–147

Chong ZZ, Lin SH, Maiese K (2004) The NAD+ precursor nicotinamide governs neuronal survival during oxidative stress through protein kinase B coupled to FOXO3a and mitochondrial membrane potential. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism 24:728–743

Ding HG, Deng YY, Yang RQ, Wang QS, Jiang WQ, Han YL, Huang LQ, Wen MY, Zhong WH, Li XS, Yang F, Zeng HK (2018) Hypercapnia induces IL-1beta overproduction via activation of NLRP3 inflammasome: implication in cognitive impairment in hypoxemic adult rats. J Neuroinflammation 15:4

DiPalma JR, Thayer WS (1991) Use of niacin as a drug. Annu Rev Nutr 11:169–187

Dragun P, Makarewicz D, Wojcik L, Ziemka-Nalecz M, Slomka M, Zalewska T (2008) Matrix metaloproteinases activity during the evolution of hypoxic-ischemic brain damage in the immature rat. The effect of 1-methylnicotinamide (MNA). Journal of Physiology and Pharmacology : an Official Journal of the Polish Physiological Society 59:441–455

Echeverria V, Yarkov A, Aliev G (2016) Positive modulators of the alpha7 nicotinic receptor against neuroinflammation and cognitive impairment in Alzheimer's disease. Prog Neurobiol 144:142–157

Erb C, Seidel A, Frank H, Platt KL, Oesch F, Klein J (1999) Formation of N-methylnicotinamide in the brain from a dihydropyridine-type prodrug: effect on brain choline. Biochem Pharmacol 57:681–684

Frost JL, Le KX, Cynis H, Ekpo E, Kleinschmidt M, Palmour RM, Ervin FR, Snigdha S, Cotman CW, Saido TC, Vassar RJ, St George-Hyslop P, Ikezu T, Schilling S, Demuth HU, Lemere CA (2013) Pyroglutamate-3 amyloid-beta deposition in the brains of humans, non-human primates, canines, and Alzheimer disease-like transgenic mouse models. Am J Pathol 183:369–381

Garcez ML, Mina F, Bellettini-Santos T, da Luz AP, Schiavo GL, Macieski JMC, Medeiros EB, Marques AO, Magnus NQ, Budni J (2018) The involvement of NLRP3 on the effects of minocycline in an AD-like pathology induced by beta-amyloid oligomers administered to mice. Mol Neurobiol

Huang Y, Zhao Z, Wei X, Zheng Y, Yu J, Zheng J, Wang L (2016) Long-term trihexyphenidyl exposure alters neuroimmune response and inflammation in aging rat: relevance to age and Alzheimer's disease. J Neuroinflammation 13:175

Hurley MJ, Deacon RMJ, Beyer K, Ioannou E, Ibáñez A, Teeling JL, Cogram P (2018) The long-lived Octodon degus as a rodent drug discovery model for Alzheimer's and other age-related diseases. Pharmacol Ther 188:36–44

Je JH, Lee JY, Jung KJ, Sung B, Go EK, Yu BP, Chung HY (2004) NF-kappaB activation mechanism of 4-hydroxyhexenal via NIK/IKK and p38 MAPK pathway. FEBS Lett 566:183–189

Jiao H, Guan F, Yang B, Li J, Song L, Hu X, Du Y (2012) Human amniotic membrane derived-mesenchymal stem cells induce C6 glioma apoptosis in vivo through the Bcl-2/caspase pathways. Mol Biol Rep 39:467–473

Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, Zahoor H, Saeed D, Natteru PA, Iyer S, Zaheer A (2017) Brain and peripheral atypical inflammatory mediators potentiate Neuroinflammation and neurodegeneration. Front Cell Neurosci 11:216

Kerr JS, Adriaanse BA, Greig NH, Mattson MP, Cader MZ, Bohr VA, Fang EF (2017) Mitophagy and Alzheimer's disease: cellular and molecular mechanisms. Trends Neurosci 40:151–166

Khan M, Im YB, Shunmugavel A, Gilg AG, Dhindsa RK, Singh AK, Singh I (2009) Administration of S-nitrosoglutathione after traumatic brain injury protects the neurovascular unit and reduces secondary injury in a rat model of controlled cortical impact. J Neuroinflammation 6:32

Kimura R, Devi L, Ohno M (2010) Partial reduction of BACE1 improves synaptic plasticity, recent and remote memories in Alzheimer's disease transgenic mice. J Neurochem 113:248–261

Kuchmerovska T, Shymanskyy I, Chlopicki S, Klimenko A (2010) 1-methylnicotinamide (MNA) in prevention of diabetes-associated brain disorders. Neurochem Int 56:221–228

Lai J, Hu M, Wang H, Hu M, Long Y, Miao MX, Li JC, Wang XB, Kong LY, Hong H (2014) Montelukast targeting the cysteinyl leukotriene receptor 1 ameliorates Abeta1-42-induced memory impairment and neuroinflammatory and apoptotic responses in mice. Neuropharmacology 79:707–714

Lawrence T, Gilroy DW, Colville-Nash PR, Willoughby DA (2001) Possible new role for NF-kappaB in the resolution of inflammation. Nat Med 7:1291–1297

Li F, Chong ZZ, Maiese K (2006) Cell life versus cell longevity: the mysteries surrounding the NAD+ precursor nicotinamide. Curr Med Chem 13:883–895

Lin S-J, Guarente L (2003) Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. Curr Opin Cell Biol 15:241–246

Lin N, Xiong L-L, R-p Z, Zheng H, Wang L, Qian Z-Y, Zhang P, Z-w C, Gao F-B, Wang T-H (2016) Injection of Aβ1-40 into hippocampus induced cognitive lesion associated with neuronal apoptosis and multiple gene expressions in the tree shrew. Apoptosis : an International Journal on Programmed Cell Death 21:621–640

Lin JR, Fang SC, Tang SS, Hu M, Long Y, Ghosh A, Sun HB, Kong LY, Hong H (2017) Hippocampal CysLT1R knockdown or blockade represses LPS-induced depressive behaviors and neuroinflammatory response in mice. Acta Pharmacol Sin 38:477–487

Liu D, Pitta M, Jiang H, Lee JH, Zhang G, Chen X, Kawamoto EM, Mattson MP (2013) Nicotinamide forestalls pathology and cognitive decline in Alzheimer mice: evidence for improved neuronal bioenergetics and autophagy procession. Neurobiol Aging 34:1564–1580

Liu H, Wang J, Wang J, Wang P, Xue Y (2015) Paeoniflorin attenuates Abeta1-42-induced inflammation and chemotaxis of microglia in vitro and inhibits NF-kappaB- and VEGF/Flt-1 signaling pathways. Brain Res 1618:149–158

Lowry JR, Klegeris A (2018) Emerging roles of microglial Cathepsins in neurodegenerative disease. Brain Res Bull 139:144–156

Magni G, Amici A, Emanuelli M, Orsomando G, Raffaelli N, Ruggieri S (2004) Enzymology of NAD+ homeostasis in man. Cellular and Molecular Life Sciences : CMLS 61:19–34

Maurice T, Lockhart BP, Privat A (1996) Amnesia induced in mice by centrally administered beta-amyloid peptides involves cholinergic dysfunction. Brain Res 706:181–193

Nasoohi S, Ismael S, Ishrat T (2018) Thioredoxin-interacting protein (TXNIP) in cerebrovascular and neurodegenerative diseases: regulation and implication. Mol Neurobiol

Nitta A, Fukuta T, Hasegawa T, Nabeshima T (1997) Continuous infusion of beta-amyloid protein into the rat cerebral ventricle induces learning impairment and neuronal and morphological degeneration. Jpn J Pharmacol 73:51–57

Pandey R, Rai V, Mishra J, Mandrah K, Kumar Roy S, Bandyopadhyay S (2017) From the cover: arsenic induces hippocampal neuronal apoptosis and cognitive impairments via an up-regulated BMP2/Smad-dependent reduced BDNF/TrkB signaling in rats. Toxicological Sciences : an Official Journal of the Society of Toxicology 159: 137–158

Pfau ML, Russo SJ (2016) Neuroinflammation regulates cognitive impairment in socially defeated mice. Trends Neurosci 39:353–355

Piirainen S, Youssef A, Song C, Kalueff AV, Landreth GE, Malm T, Tian L (2017) Psychosocial stress on neuroinflammation and cognitive dysfunctions in Alzheimer's disease: the emerging role for microglia? Neurosci Biobehav Rev 77:148–164

Przygodzki T, Kazmierczak P, Sikora J, Watala C (2010) 1-methylnicotinamide effects on the selected markers of endothelial function, inflammation and haemostasis in diabetic rats. Eur J Pharmacol 640:157–162

Pujadas L, Rossi D, Andres R, Teixeira CM, Serra-Vidal B, Parcerisas A, Maldonado R, Giralt E, Carulla N, Soriano E (2014) Reelin delays amyloid-beta fibril formation and rescues cognitive deficits in a model of Alzheimer's disease. Nat Commun 5:3443

Qian Y, Yin J, Hong J, Li G, Zhang B, Liu G, Wan Q, Chen L (2016) Neuronal seipin knockout facilitates Abeta-induced neuroinflammation and neurotoxicity via reduction of PPARgamma in hippocampus of mouse. J Neuroinflammation 13:145

Rahimifard M, Maqbool F, Moeini-Nodeh S, Niaz K, Abdollahi M, Braidy N, Nabavi SM, Nabavi SF (2017) Targeting the TLR4 signaling pathway by polyphenols: a novel therapeutic strategy for neuroinflammation. Ageing Res Rev 36:11–19

Rubio-Perez JM, Morillas-Ruiz JM (2012) A review: inflammatory process in Alzheimer's disease, role of cytokines. TheScientificWorldJournal 2012:756357

Ruiz-Vela A, Opferman JT, Cheng EHY, Korsmeyer SJ (2005) Proapoptotic BAX and BAK control multiple initiator caspases. EMBO Rep 6:379–385

Russo I, Caracciolo L, Tweedie D, Choi SH, Greig NH, Barlati S, Bosetti F (2012) 3,6′-Dithiothalidomide, a new TNF-alpha synthesis inhibitor, attenuates the effect of Abeta1-42 intracerebroventricular injection on hippocampal neurogenesis and memory deficit. J Neurochem 122: 1181–1192

Sethi G, Sung B, Aggarwal BB (2008) Nuclear factor-kappaB activation: from bench to bedside. Exp Biol Med 233:21–31

Slomka M, Zieminska E, Lazarewicz J (2008a) Nicotinamide and 1-methylnicotinamide reduce homocysteine neurotoxicity in primary cultures of rat cerebellar granule cells. Acta Neurobiol Exp 68:1–9

Slomka M, Zieminska E, Salinska E, Lazarewicz JW (2008b) Neuroprotective effects of nicotinamide and 1-methylnicotinamide in acute excitotoxicity in vitro. Folia Neuropathol 46:69–80

Song D, Jiang X, Liu Y, Sun Y, Cao S, Zhang Z (2018) Asiaticoside attenuates cell growth inhibition and apoptosis induced by Abeta1-42 via inhibiting the TLR4/NF-kappaB signaling pathway in human brain microvascular endothelial cells. Front Pharmacol 9:28

Sternak M, Khomich TI, Jakubowski A, Szafarz M, Szczepanski W, Bialas M, Stojak M, Szymura-Oleksiak J, Chlopicki S (2010) Nicotinamide N-methyltransferase (NNMT) and 1-methylnicotinamide (MNA) in experimental hepatitis induced by concanavalin a in the mouse. Pharmacological Reports : PR 62:483–493

Sternak M, Jakubowski A, Czarnowska E, Slominska EM, Smolenski RT, Szafarz M, Walczak M, Sitek B, Wojcik T, Jasztal A, Kaminski K, Chlopicki S (2015) Differential involvement of IL-6 in the early and late phase of 1-methylnicotinamide (MNA) release in Concanavalin A-induced hepatitis. Int Immunopharmacol 28: 105–114

Stewart CR, Stuart LM, Wilkinson K, van Gils JM, Deng J, Halle A, Rayner KJ, Boyer L, Zhong R, Frazier WA, Lacy-Hulbert A, El Khoury J, Golenbock DT, Moore KJ (2010) CD36 ligands promote sterile inflammation through assembly of a toll-like receptor 4 and 6 heterodimer. Nat Immunol 11:155–161

Takeda K, Akira S (2004) TLR signaling pathways. Semin Immunol 16: 3–9

Tanaka Y, Kume S, Araki H, Nakazawa J, Chin-Kanasaki M, Araki S, Nakagawa F, Koya D, Haneda M, Maegawa H, Uzu T (2015) 1-Methylnicotinamide ameliorates lipotoxicity-induced oxidative stress and cell death in kidney proximal tubular cells. Free Radic Biol Med 89:831–841

Tang SS, Wang XY, Hong H, Long Y, Li YQ, Xiang GQ, Jiang LY, Zhang HT, Liu LP, Miao MX, Hu M, Zhang TT, Hu W, Ji H, Ye FY (2013) Leukotriene D4 induces cognitive impairment through enhancement of CysLT(1) R-mediated amyloid-beta generation in mice. Neuropharmacology 65:182–192

Tang SS, Hong H, Chen L, Mei ZL, Ji MJ, Xiang GQ, Li N, Ji H (2014a) Involvement of cysteinyl leukotriene receptor 1 in Abeta1-42-induced neurotoxicity in vitro and in vivo. Neurobiol Aging 35: 590–599

Tang SS, Ji MJ, Chen L, Hu M, Long Y, Li YQ, Miao MX, Li JC, Li N, Ji H, Chen XJ, Hong H (2014b) Protective effect of pranlukast on Abeta(1)(−)(4)(2)-induced cognitive deficits associated with down-regulation of cysteinyl leukotriene receptor 1. Int J Neuropsychopharmacol 17:581–592

Tang R, Lin YM, Liu HX, Wang ES (2018) Neuroprotective effect of docosahexaenoic acid in rat traumatic brain injury model via regulation of TLR4/NF-kappa B signaling pathway. Int J Biochem Cell Biol 99:64–71

Tusi SK, Ansari N, Amini M, Amirabad AD, Shafiee A, Khodagholi F (2010) Attenuation of NF-kappaB and activation of Nrf2 signaling by 1,2,4-triazine derivatives, protects neuron-like PC12 cells against apoptosis. Apoptosis : an International Journal on Programmed Cell Death 15:738–751

Ungerstedt JS, Blomback M, Soderstrom T (2003) Nicotinamide is a potent inhibitor of proinflammatory cytokines. Clin Exp Immunol 131:48–52

Wang WY, Tan MS, Yu JT, Tan L (2015) Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Annals of Translational Medicine 3:136

Wang SW, Liu DQ, Zhang LX, Ji M, Zhang YX, Dong QX, Liu SY, Xie XX, Liu RT (2017) A vaccine with Abeta oligomer-specific mimotope attenuates cognitive deficits and brain pathologies in transgenic mice with Alzheimer's disease. Alzheimers Res Ther 9:41

Williams AC, Cartwright LS, Ramsden DB (2005) Parkinson's disease: the first common neurological disease due to auto-intoxication? QJM : Monthly Journal of the Association of Physicians 98:215–226

Wyss-Coray T (2006) Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 12:1005–1015

Xie X, Liu H, Wang Y, Zhou Y, Yu H, Li G, Ruan Z, Li F, Wang X, Zhang J (2016) Nicotinamide N-methyltransferase enhances resistance to 5-fluorouracil in colorectal cancer cells through inhibition of the ASK1-p38 MAPK pathway. Oncotarget 7:45837–45848

Ye SM, Johnson RW (2001) Regulation of interleukin-6 gene expression in brain of aged mice by nuclear factor kappaB. J Neuroimmunol 117:87–96

Zhang CT, Lin JR, Wu F, Ghosh A, Tang SS, Hu M, Long Y, Sun HB, Hong H (2016) Montelukast ameliorates streptozotocin-induced cognitive impairment and neurotoxicity in mice. Neurotoxicology 57:214–222

Zhang Z, Li X, Li D, Luo M, Li Y, Song L, Jiang X (2017) Asiaticoside ameliorates beta-amyloid-induced learning and memory deficits in rats by inhibiting mitochondrial apoptosis and reducing inflammatory factors. Experimental and Therapeutic Medicine 13:413–420