

Milrinone Ameliorates the Neuroinflammation and Memory Function of Alzheimer's Disease in an APP/PS1 Mouse Model

Qingyou Chen¹

Yue Yin²

Li Li¹

Yanjiao Zhang¹

Wei He¹

Yan Shi³

¹Department of Neurology, The Third Affiliated Hospital of Qiqihar Medical University, Qiqihar City, Heilongjiang Province, 161000, People's Republic of China; ²Department of Science and Education, The Third Affiliated Hospital of Qiqihar Medical University, Qiqihar City, Heilongjiang Province, 161000, People's Republic of China; ³College of Medical Technology, Qiqihar Medical University, Qiqihar City, Heilongjiang Province, 161000, People's Republic of China

Purpose: Alzheimer's disease (AD) is a complex neurodegenerative disorder, which is characterized by memory loss and cognitive deficits. The neuroprotective role of milrinone on the injury of spinal cord or cerebral ischemia-reperfusion has been confirmed. However, the accurate function of milrinone on AD pathogeny is still unclear.

Methods: APP/PS1 transgenic mouse was used to explore the role of milrinone in behaviour tests, and the effects on histopathologic features of AD such as the formation of neuronal amyloid- β (A β) plaque, microglial activation, tau protein hyperphosphorylation, oxidative stress, and neuroinflammation. Lipopolysaccharide (LPS)/A β -treated BV-2 cells were used to understand the anti-inflammation mechanism of milrinone on AD in vitro.

Results: Our in vivo results showed that milrinone ameliorates the memory functions of AD mice. Meanwhile, milrinone reduced A β deposits, repressed microglial activation and tau protein hyperphosphorylation, attenuated the oxidative stress, and decreased the levels of inflammatory cytokines. The in vitro results demonstrated that milrinone could inhibit the secretion of interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α via regulation of NLRP3 inflammasomes and TLR4/MyD88/NF- κ B signalling pathway.

Conclusion: Overall, milrinone could ameliorate the memory loss and cognitive deficits through repressing the multiple pathological processes of AD, suggesting that milrinone may be an underlying and effective drug for treating AD clinically.

Keywords: milrinone, Alzheimer's disease, neuroinflammation, memory loss, cognitive deficit

Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder, which commonly results in hypomnesia and cognitive deficits.¹ There are approximately 50 million people suffering from AD in 2017 all over the world, and this number is constantly increasing in the next 30 years.² The histopathologic characterizations of AD include a series of interactive processes such as neuronal amyloid- β (A β) plaque formation, microglial activation, tau protein hyperphosphorylation, and neuroinflammation.^{3,4} A β deposits are considered to enrich the activation of microglial cells.⁵ The activated microglial cell is a crucial neuroinflammation factor to accelerate NLRP3 inflammasomes formation⁶ and promotes the secretion of inflammatory cytokines such as IL-1 β and IL-6, which in turn increases the level of A β plaque.^{7,8} Meanwhile, tau hyperphosphorylation especially the sites threonine 231 (T231) and serine 396 (S396) phosphorylated prematurely is an important hallmark for the occurrence of AD.^{9,10}

Correspondence: Yan Shi
College of Medical Technology, Qiqihar Medical University, No. 333, Bukui Street, Jianhua District, Qiqihar City, Heilongjiang Province, 161000, People's Republic of China
Tel +86-0452-2697374
Email shiyan357@126.com

Currently, only few drugs including Tacrine, Donepezil, Galantamine, Memantine, and Rivastigmine are confirmed to be used for treatment with AD.^{11–15} However, these therapies are generally used to control symptoms rather than alter the course of AD, or even lead to some severe side effects such as hepatotoxicity, gastrointestinal fatigue, and muscle cramps.^{11–15} Therefore, exploring clinical drugs based on inhibition of AD pathological features is particularly important for improving AD.

Milrinone is a kind of bipyridine with vasodilator properties, which is generally used in the therapies for cardiopulmonary diseases.^{16,17} For instance, Qasim and Jain revealed that milrinone can ameliorate persistent pulmonary hypertension in newborn through the great improvement for cardiac function and the reduction for pulmonary vascular resistance.¹⁶ Tang et al performed a meta-analysis for the function of milrinone on patients with acute heart failure (AHF) or acute myocardial infarction (AMI); they uncovered that milrinone may be an effectively clinical drug for the treatment of AHF and AML.¹⁷ More importantly, growing evidences uncovered the clinical use of milrinone in neurological and neurocritical care patients, including subarachnoid hemorrhage (SAH) and stroke.^{18,19} For example, a clinical study of the application of milrinone on SAH patients in Montreal Neurological Hospital has indicated that intravenous milrinone infusion and the maintenance of homeostasis is simple to use and requires less intensive monitoring and resources than the standard hypertension, hypervolemia, and hemodilution (triple-H) therapy for SAH.¹⁸ Labeyrie et al found that intravenous milrinone can effectively decrease the incidence of stroke.¹⁹ In addition, the neuroprotective role of milrinone on nerve or brain injury-related diseases has been also confirmed in animal models.^{20–22} Arac et al reported that milrinone represses neurocytes apoptosis and inflammation in spinal cord injury (SCI) rats, thereby making valuable contributions on the function recovery of spinal cord tissues.²⁰ Choi et al made the comparisons among the effects of milrinone, sodium nitroprusside, and nitroglycerin on patients with cerebral perfusion injury, and found that milrinone treatment is more helpful for the cognitive function recovery relative to the other two treatments.²¹ Saklani et al further explored the protective effect of milrinone on cerebral ischemia-reperfusion injury (CI/RI) mice, and indicated that milrinone protect against memory loss after CI/RI through regulation of calcium level.²² As a novel drug

treatment with brain diseases, however, there are relatively rare studies on the effects of milrinone for AD therapies.

In this study, the function of milrinone on the malignant behaviors of AD and its detailed action mechanism on AD pathogenesis were investigated. Our results may provide an underlying drug to attenuate AD and not just control its clinical symptoms.

Materials and Methods

AD Mouse Model and Drug Treatment

All animal experiments in this study were in strict accordance with the protocols stated in the Guide for the Care and Use of Laboratory Animals and approved by the ethical committee of The Third Affiliated Hospital of Qiqihar Medical University. Female APP/PS1 mice (30 weeks) and age-matched female wild-type C57BL/6L mice (control group) were procured from Jackson Laboratory. The mice were allowed to adapt to the laboratory environment for 2 weeks before testing. Afterwards, the APP/PS1 mice (32 weeks) were further assigned into two groups randomly: the APP/PS1 and APP/PS1 + M groups (n = 6). The mice in APP/PS1 + M group were injected with milrinone (i.p., 0.5 mg/kg),²⁰ while the mice in the control and APP/PS1 groups were intraperitoneally injected with the equal saline. All mice were treated once a day for a month.

Passive Avoidance Test

Passive avoidance test (PAT) was performed according to the previous study.²³ The experimental apparatus used in this study was purchased from Taimeng Tech (Chengdu, China), which was divided into two independent compartments (one for light, and one for dark) connected together via an automatic guillotine door. This test included a training test and a formal test (one day after the training). For training test, the mice of the above groups were initially placed into the light compartment to habituate for 180 sec, and then the guillotine door was opened to allow the mice to enter the dark compartment, in which a footshock (0.5 mA, 2 sec) was delivered. Ten sec later, the mice were removed from the dark compartment. Similar procedures were performed in the formal test except for footshock. The frequencies and latencies for the mice entering from the light compartment to the dark one were recorded with a maximum of 300 sec.

Morris Water Maze Test

As the previous study mentioned,²⁴ the Morris water maze test (MWMT) was conducted. Briefly, all the mice were initially placed into a water pool (temperature: $23 \pm 1^\circ\text{C}$, diameter: 1.5 m, height: 0.6 m) for 1 min to adapt to the environment. Subsequently, a hidden platform (immersed 1 cm below the water) was set in the middle of the platform. The mice were placed into the pool to find the platform within 60 sec. Each mouse was tested three times a day at intervals of at least 15 min. The time required to find the hidden platform was recorded as the escape latency. To assess the memory ability, the invisible platform was removed and the mice were allowed to swim freely for 60 sec. The numbers of crossing the original position of the platform were recorded. All the data were obtained using a video tracking system (Taimeng Tech).

Brain Tissues Collection

After the above behavior tests, all the mice were anaesthetized by pentobarbital sodium (i.p., 50 mg/kg) and sacrificed by decapitation. The hippocampus and cortex tissues were collected and immediately frozen at -80°C for further analysis.

Cell Culture, Grouping, and Treatments

Mouse microglial cell line BV-2 (immortalized cell) was procured from Cobioer biotech (Nanjing, China) and cultured in Dulbecco's Modified Eagle's medium (DMEM) with 5% fetal bovine serum and 1% streptomycin/penicillin at 37°C with 5% CO_2 . Milrinone was dissolved in 0.3% carboxyl methyl cellulose sodium (CMC-Na), and $\text{A}\beta_{1-42}$ was dissolved in sterile phosphate buffered saline (PBS). Afterwards, the cells were initially pretreated with different concentrations of milrinone (0, 10, 25, 50 $\mu\text{mol/L}$) for 1 h, followed by the stimulation with $\text{A}\beta_{1-42}$ (10 $\mu\text{mol/L}$) and lipopolysaccharide (LPS) (1 $\mu\text{g/mL}$). The cells treated with equal PBS were used as controls. Approximately 1 day later, the levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 were assessed by enzyme-linked immunosorbent assay (ELISA).

ELISA Analysis

According to the manufacturer's protocol, the levels of the inflammatory factors (TNF- α , IL-1 β , and IL-6) in mouse hippocampus, cortex, and BV-2 cells were measured using specific ELISA kits (MSK Biotech, Ltd., Wuhan, China), while the levels of superoxide dismutase (SOD),

malondialdehyde (MDA) and glutathione (GSH) in mouse hippocampus, and the levels of $\text{A}\beta_{40}$ and $\text{A}\beta_{42}$ in mouse hippocampus and cortex tissues were measured using the corresponding commercial assay kits (MSK Biotech).

Western Blotting Analysis

RIPA buffer containing protease inhibitors was used to extract proteins from mouse brain tissues and BV-2 cells. Protein concentrations were then determined using a BCA Protein Assay Kit (Abcam, Cambridge, UK). Protein samples were separated via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the resolved proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% bovine serum albumin at room temperature. After blocking, membranes were incubated overnight at 4°C with primary antibodies against TLR4 (1:1000; Abcam), MyD88 (1:1000; Abcam), NF- κB (p65) (1:1000; Abcam), phospho-NF- κB (p-p65) (1:1000; Cell Signaling), NLRP3 (1:1000; Abcam), ASC (1:1000; Affinity Biosciences), caspase 1 (1:1000; Affinity Biosciences), IL-1 β (1:1000; Abcam), IL-18 (1:1000; Abcam), Iba-1 (1:1000; Abcam), p-Tau (S396) (1:1000; Abcam), p-Tau (T231) (1:1000; Abcam), and GAPDH (1:1000; Abcam). Thereafter, they were washed three times with Tris-buffered saline Tween-20. Subsequently, an HRP-conjugated IgG secondary antibody (1:5000; Santa Cruz, Waltham, MA, USA) was added and membranes were incubated at room temperature for 1 h. GAPDH was used as the internal reference. An enhanced chemiluminescence detection kit (Thermo Fisher Scientific) was used to detect the bands, which were then quantified using Gel-Pro Analyzer software (version 4.0; Media Cybernetics, Silver Spring, MD, USA).

Statistical Analysis

SPSS 20.0 software (Chicago, USA) was used in analysis. One-way ANOVA, followed by Tukey's multiple comparisons test was used to assess experimental data. Data were presented as means \pm SD. P-value less than 0.05 indicated a statistically significant difference.

Results

Milrinone Ameliorates the Memory Functions of APP/PS1 Mice

To explore the therapeutic efficacy of milrinone on AD, relevant behaviour tests were performed on APP/PS1 mice. As presented in Figure 1A and B, we found that

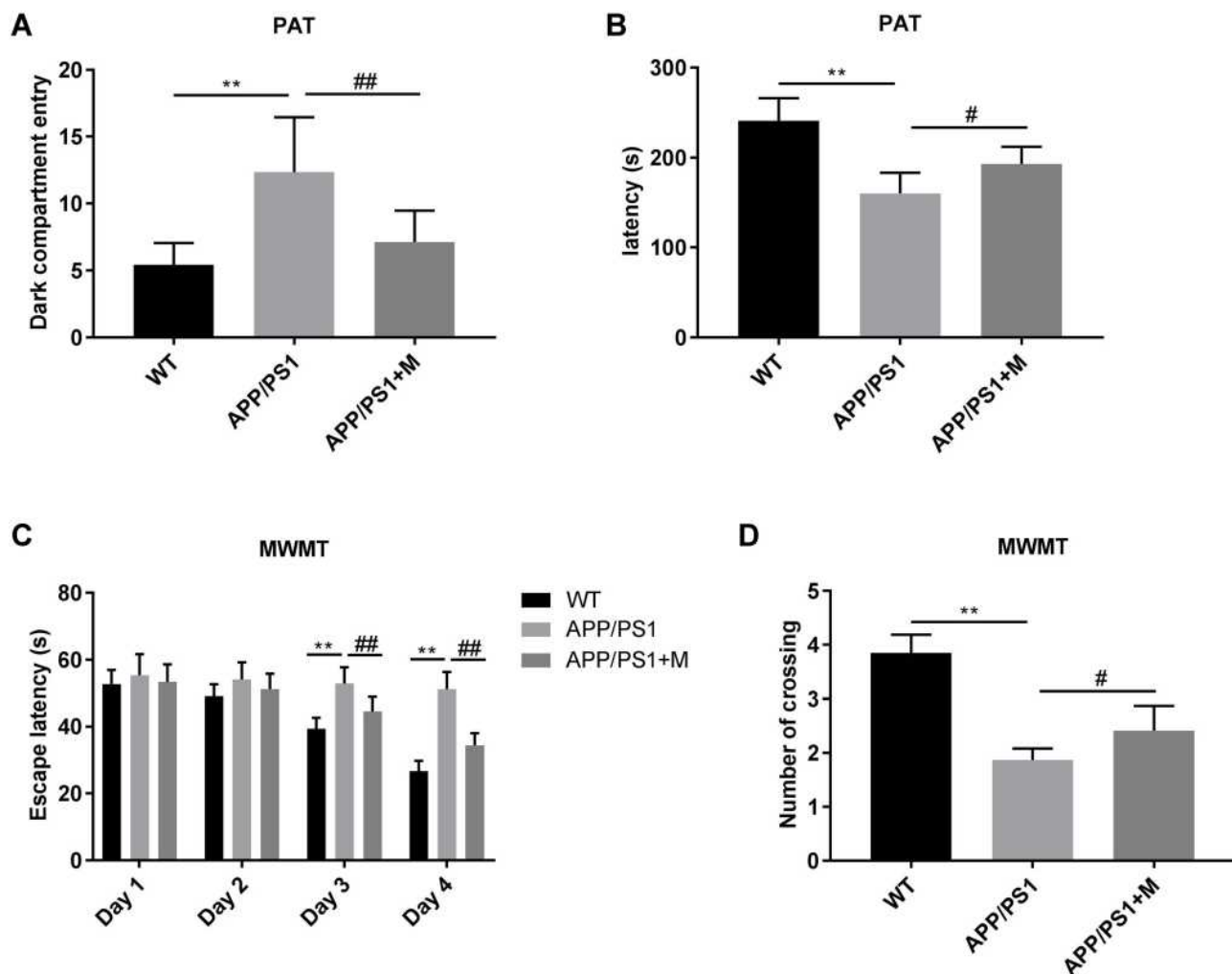


Figure 1 Milrinone ameliorates the memory functions of APP/PS1 mice. **(A)** The number of APP/PS1 mice entering the dark compartment during the passive avoidance test (PAT). **(B)** The latency of APP/PS1 mice in the PAT. **(C)** The escape latency of APP/PS1 mice in the Morris water maze test (MWMT). **(D)** The number of crossings through the original position of the platform in MWMT. ** $P < 0.01$ vs the WT group; # $P < 0.05$, ### $P < 0.01$ vs the APP/PS1 group.

APP/PS1 mice had a relatively higher frequency to enter the dark compartment and a shorter latency before entering the dark compartment than the mice of the WT group ($P < 0.01$). However, these situations were all reversed in the milrinone treatment group ($P < 0.05$). Subsequently, the results of MWMT demonstrated that there seemed no significant differences among the three aforementioned groups on day 1 and 2. From day 3 to day 4, the escape latency of APP/PS1 mice was longer compared to that of the WT mice, while milrinone treatment remarkably shortened the escape latency (Figure 1C, $P < 0.01$). Further probe experiments revealed that decreased frequency of crossing over the platform position was observed in APP/PS1 mice by contrast to the WT mice (Figure 1D, $P < 0.01$). On the contrary, it was increased in the treatment group ($P < 0.05$).

Milrinone Inhibits A β Plaque Formation, Microglia Activation, and Tau Hyperphosphorylation in APP/PS1 Mice

The formation of A β plaque is identified as an important hallmark of AD. Meanwhile, both microglia activation and tau hyperphosphorylation are also accompanied with the progression of AD. Therefore, the effects of milrinone on the above biological processes were further investigated. As illustrated in Figure 2A–D, we found that the levels of A β_{40} and A β_{42} in cortex and hippocampus of APP/PS1 mice were expected increased ($P < 0.01$). In contrast, the mice in the milrinone group had relatively low levels of A β plaque ($P < 0.05$). In addition, the level of Iba-1 (microglia marker) was also elevated in APP/PS1 mice (Figure 2E and F, $P < 0.01$), whereas it was repressed in the milrinone treatment group ($P <$

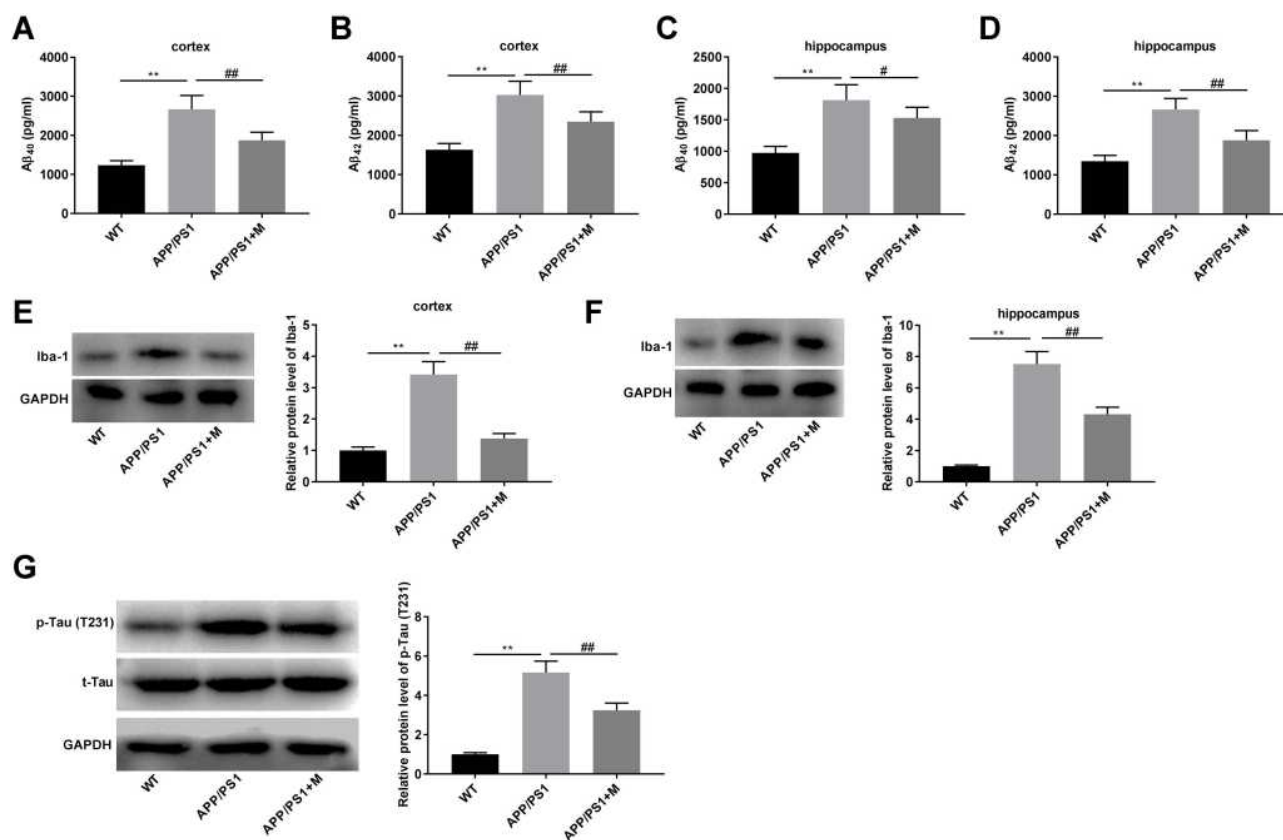


Figure 2 Milrinone inhibits the formation of A β plaque, activation of microglia, and the hyperphosphorylation of tau in APP/PS1 mice. (A) The level of A β ₄₀ in APP/PS1 mice cortex was measured by ELISA analysis. (B) The level of A β ₄₂ in APP/PS1 mice cortex was measured by ELISA analysis. (C) The level of A β ₄₀ in APP/PS1 mice hippocampus was measured by ELISA analysis. (D) The level of A β ₄₂ in APP/PS1 mice hippocampus was measured by ELISA analysis. (E) The protein level of Iba-1 in APP/PS1 mice cortex was determined by Western blot assay. (F) The protein level of Iba-1 in APP/PS1 mice hippocampus was determined by Western blot assay. (G) The protein levels of p-Tau (Ser396) and p-Tau (Thr231) in APP/PS1 mice brain tissues were determined by Western blot assay. ***P* < 0.01 vs the WT group; #*P* < 0.05, ##*P* < 0.01 vs the APP/PS1 group.

0.01). Similar patterns were observed in the protein levels of p-Tau (S396) and p-Tau (T231) (Figure 2G, *P* < 0.01).

Milrinone Attenuates the Oxidative Stress in APP/PS1 Mice

To further explore the effect of milrinone on oxidative stress, the levels of MDA, SOD, and GSH-Px were measured by Western blotting. We found that MDA level was increased, and both SOD and GSH-Px were decreased in hippocampus of APP/PS1 mice (Figure 3A–C, *P* < 0.01). Unsurprisingly, these situations were reversed in APP/PS1 mice treatment with milrinone (*P* < 0.05).

Milrinone Represses the Inflammatory Responses in APP/PS1 Mice

Inflammatory responses occur in the development of AD. We then assessed the function of milrinone on AD inflammation. As shown in Figure 4A–F, high levels of IL-1 β , IL-6, and TNF- α were found in cortex and hippocampus

tissues of APP/PS1 mice (*P* < 0.01), whereas milrinone repressed the release of these inflammatory cytokines from the damaged brain tissues (*P* < 0.05).

Milrinone Diminishes the Secretion of Inflammatory Cytokines and Inhibits the Formation of NLRP3 Inflammasomes in BV-2 Cells

As presented in Figure 5A–C, different concentrations of milrinone (0, 10, 25, 50 μ mol/L) were assigned to explore its effect on inflammation in BV-2 cells. We found that LPS/A β significantly promoted inflammation levels of BV-2 cells (*P* < 0.01). Different milrinone concentrations reduced the secretion of inflammatory cytokines to a certain extent (*P* < 0.05). Meanwhile, we further demonstrated that there were no significant differences between the LPS/A β and LPS/A β + M10 group, as well as between the LPS/A β + M25 and LPS/A β + M50 group in inflammation level. Therefore, M25 was used to perform the subsequent trails. NLRP3

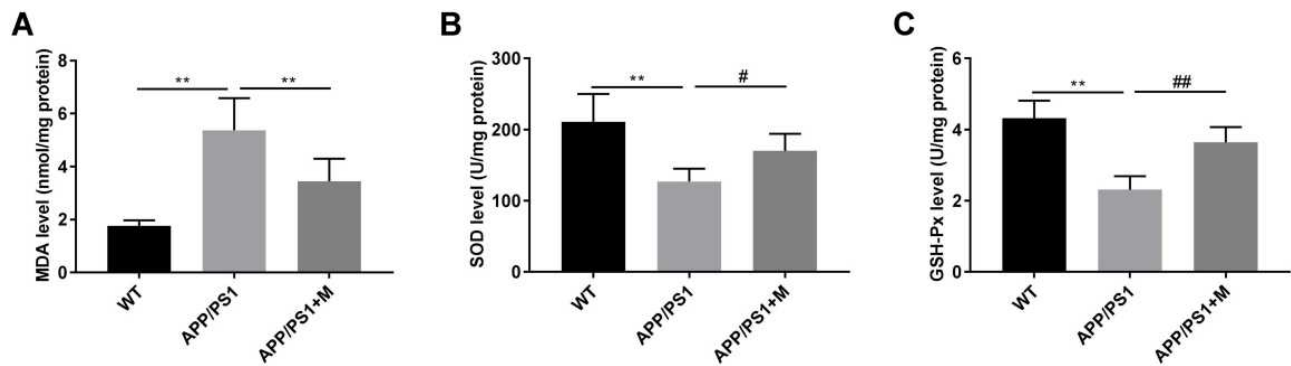


Figure 3 Milrinone attenuates the oxidative stress in APP/PS1 mice. **(A)** The level of MDA in APP/PS1 mice brain tissues was measured by ELISA analysis. **(B)** The level of SOD in APP/PS1 mice brain tissues was measured by ELISA analysis. **(C)** The level of GSH-Px in APP/PS1 mice brain tissues was measured by ELISA analysis. ** $P < 0.01$ vs the WT group; # $P < 0.05$, ### $P < 0.01$ vs the APP/PS1 group.

inflammasomes are commonly strongly correlated with neuroinflammation.^{25,26} The levels of NLRP3 inflammasomes-related proteins (ASC, NLRP3, active caspase 1, IL-1 β , and IL-18) were assessed in LPS/A β -induced BV-2 cells. We found that the levels of the aforementioned proteins were all elevated in the LPS/A β group (Figure 5D–I, $P < 0.01$). In the LPS/A β + M25 group, however, the levels of ASC,

NLRP3, active caspase 1, IL-1 β , and IL-18 were suppressed compared to those of the LPS/A β group ($P < 0.05$).

Milrinone Inactivates the TLR4/MyD88/NF- κ B Signaling Pathway in BV-2 Cells

Finally, the interaction between milrinone and TLR4/MyD88/NF- κ B signaling pathway was investigated due

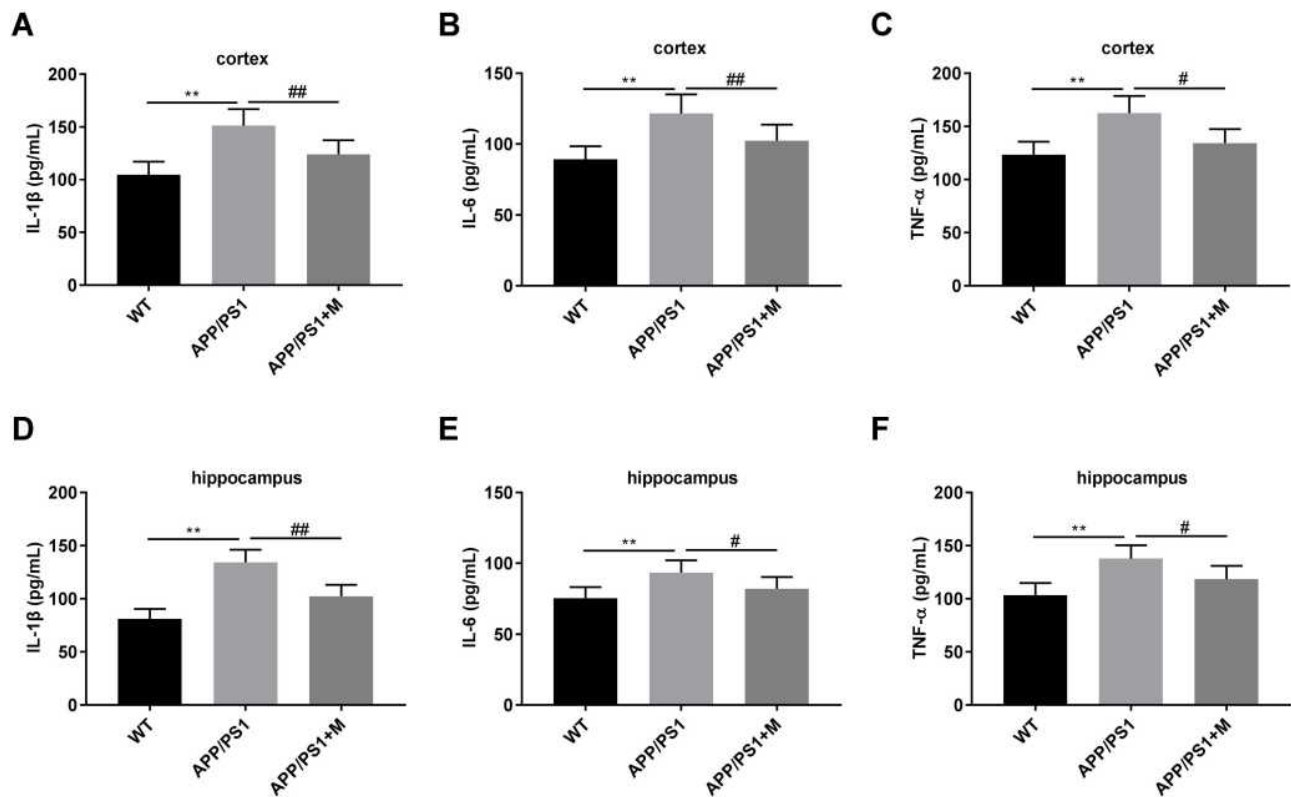


Figure 4 Milrinone represses the inflammatory responses in APP/PS1 mice. **(A)** The level of IL-1 β in APP/PS1 mice cortex was measured by ELISA analysis. **(B)** The level of IL-6 in APP/PS1 mice cortex was measured by ELISA analysis. **(C)** The level of TNF- α in APP/PS1 mice cortex was measured by ELISA analysis. **(D)** The level of IL-1 β in APP/PS1 mice hippocampus was measured by ELISA analysis. **(E)** The level of IL-6 in APP/PS1 mice hippocampus was measured by ELISA analysis. **(F)** The level of TNF- α in APP/PS1 mice hippocampus was measured by ELISA analysis. ** $P < 0.01$ vs the WT group; # $P < 0.05$, ### $P < 0.01$ vs the APP/PS1 group.

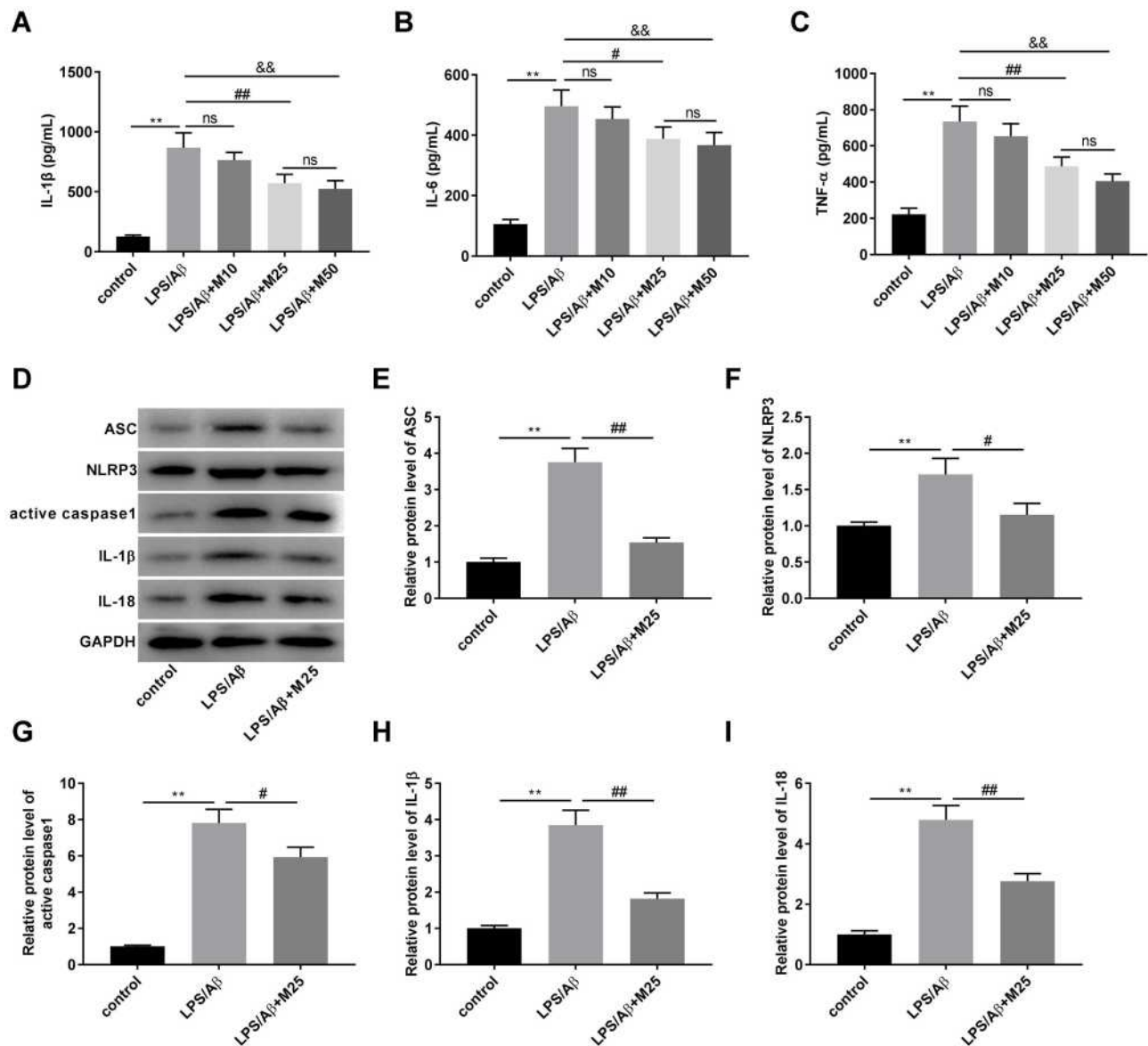


Figure 5 Milrinone diminishes the secretion of inflammatory cytokines and inhibits the formation of NLRP3 inflammasomes. (A) The level of IL-1 β in LPS/A β -treated BV-2 cells with different concentrations of milrinone (0, 10, 25, 50 μ mol/L) was measured by ELISA analysis. (B) The level of IL-6 in LPS/A β -treated BV-2 cells with different concentrations of milrinone (0, 10, 25, 50 μ mol/L) was measured by ELISA analysis. (C) The level of TNF- α in LPS/A β -treated BV-2 cells with different concentrations of milrinone (0, 10, 25, 50 μ mol/L) was measured by ELISA analysis. (D) The Western blot assay images for the levels of ASC, NLRP3, active caspase 1, IL-1 β , and IL-18 in LPS/A β -treated BV-2 cells. (E) The protein level of ASC in LPS/A β -treated BV-2 cells was measured by Western blot assay. (F) The protein level of NLRP3 in LPS/A β -treated BV-2 cells was measured by Western blot assay. (G) The protein level of active caspase 1 in LPS/A β -treated BV-2 cells was measured by Western blot assay. (H) The protein level of IL-1 β in LPS/A β -treated BV-2 cells was measured by Western blot assay. (I) The protein level of IL-18 in LPS/A β -treated BV-2 cells was measured by Western blot assay. ** P < 0.01 vs the control group; # P < 0.05, ### P < 0.01, && P < 0.01 vs the LPS/A β group. ns: not significant.

to the important role of this pathway in microglial mediated inflammation of brain injury. As shown in Figure 6A–D, we discovered that the levels of TLR4, MyD88, and p-p65/p65 were upregulated in the LPS/A β group (P < 0.01), whereas milrinone reversed the increased levels of TLR4, MyD88, and p-p65/p65 caused by LPS/A β inducement (P < 0.01).

Discussion

Alzheimer's disease mainly occurs in the aged population and its important characterizations are memory loss and cognitive decline.¹ The interactions among A β plaque, microglial activation, tau protein hyperphosphorylation, and neuroinflammation were the main histopathologic features that affect the progression of AD.^{3,4} This study

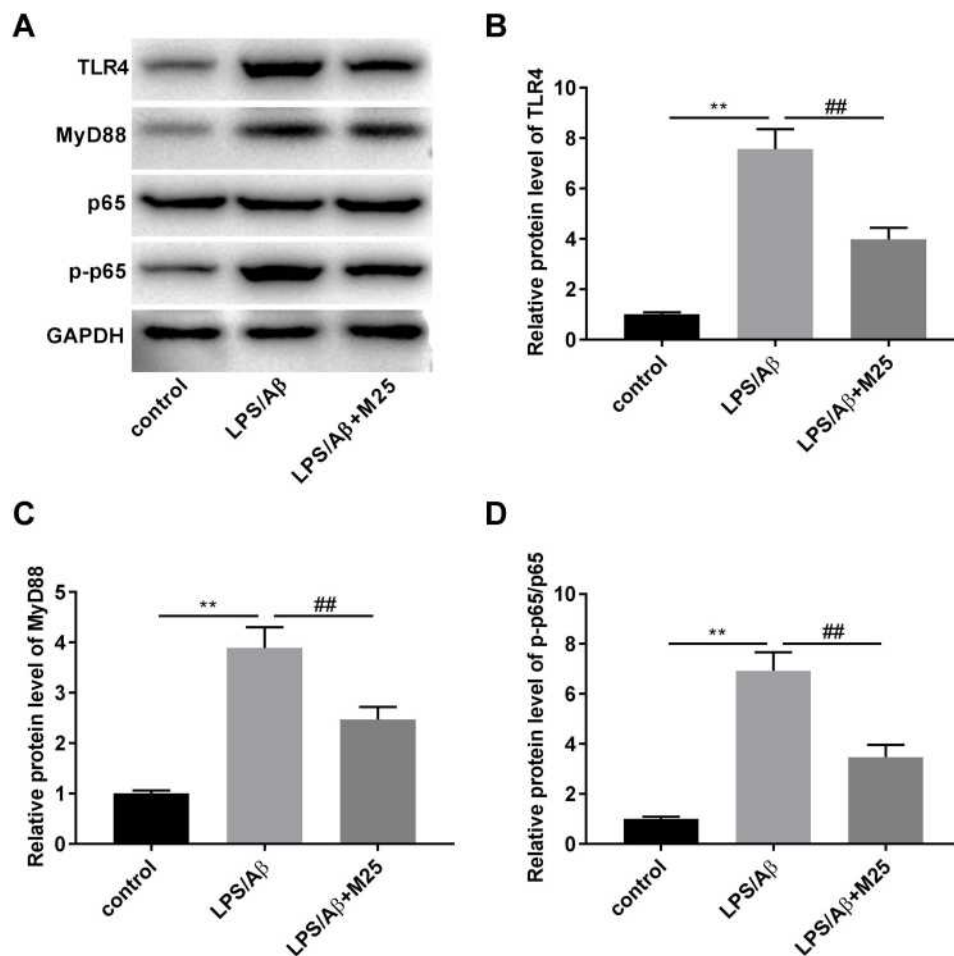


Figure 6 Milrinone inactivates the TLR4/MyD88/NF- κ B signaling pathway in BV-2 cells. **(A)** The Western blot assay images for the levels of TLR4, MyD88, p65, and p-p65 in LPS/A β -treated BV-2 cells. **(B)** The protein level of TLR4 in LPS/A β -treated BV-2 cells was measured by Western blot assay. **(C)** The protein level of MyD88 in BV-2 cells was measured by Western blot assay. **(D)** The protein level of p-p65/p65 in BV-2 cells was measured by Western blot assay. ** $P < 0.01$ vs the control group. ## $P < 0.01$ vs the LPS/A β group.

focused on the regulatory mechanisms of milrinone on A β deposits, microglial activation, tau phosphorylation, oxidative stress, and inflammation, which demonstrated that milrinone could ameliorate memory deficits and cognitive decline caused by AD.

APP/PS1 transgenic mouse was generally used for AD researches.^{27,28} In this study, the analyses for PAT and MWMT demonstrated that milrinone treatment ameliorated cognitive deficits in learning and memory function in APP/PS1 mice. We speculated milrinone may be an effective drug for AD therapy. The enrichment of A β deposits is the initial changes in brain tissues after AD and aggravates the toxicity to central neural system.^{29,30} Numerous studies further confirmed that inhibition of A β deposit levels in brain tissues is beneficial to the cognitive restoration of AD.^{27,28,31,32} In our study, relatively high levels of A β_{40} and A β_{42} were found in both cortex and hippocampus of APP/PS1 mice, whereas they were

repressed by injection of milrinone, suggesting that milrinone suppresses the accumulation of A β deposits in APP/PS1 mice.

Previous study has revealed that the accumulated A β triggered the activation of microglia, in which process is accompanied with the release of inflammatory cytokines.³³ Therefore, inflammation reactions in APP/PS1 mice with or without milrinone treatment were initially studied. We discovered that the levels of IL-1 β , IL-6, and TNF- α in LPS/A β -treated BV-2 cells and APP/PS1 mice brain tissues (cortex and hippocampus) were elevated. By contrast, milrinone restrained the inflammation responses. Similarly, a recent study on milrinone for SCI therapy has indicated that the inflammation caused by SCI can be attenuated by milrinone treatment.²⁰ The results implied that milrinone may function as an anti-inflammatory role in AD pathogenesis. It has been confirmed that NLRP3 inflammasome serves as a vital factor in the progression of neuroinflammation.^{34–36} We further speculated

NLRP3 inflammasomes-related proteins (ASC, NLRP3, active caspase 1, IL-1 β , and IL-18) may be also inhibited by milrinone. The experimental data that the levels of ASC, NLRP3, active caspase 1, IL-1 β , and IL-18 in LPS/A β -treated BV-2 cells were suppressed by milrinone inducement validated this hypothesis. In addition, numerous studies demonstrated that A β deposits can enter the mitochondria to induce oxidative stress and result in toxicity to the neurocytes.^{37,38} Interestingly, in this study, we found milrinone reversed the toxic effects of oxidative stress on AD mice, which process may attenuate the oxidative stress damages to neurons. Furthermore, the sites T231 and S396 phosphorylated earlier are considered as important hallmarks for the occurrence of AD.^{9,10} In the current study, the levels of p-Tau (S396) and p-Tau (T231) were unsurprisingly elevated in the brain tissues of AD mice, while were restrained by milrinone. Meanwhile, we also uncovered that the protein level of Iba-1, a marker of microglial activation, was inhibited by milrinone, pointing out that milrinone is an inhibitor for the activation of microglia. All the above results suggested that milrinone may ameliorate AD via repression of neuroinflammation, oxidative stress, and tau hyperphosphorylation as well as regulation of A β burden and microgliosis.

The activation of TLR4/MyD88/NF- κ B signalling pathway is closely correlated with several inflammation diseases, such as coronary microembolization,³⁹ acute lung injury,⁴⁰ and knee osteoarthritis.⁴¹ In the course of AD, the occurrence of neuroinflammation has been confirmed in our above conclusions. Therefore, we speculated TLR4/MyD88/NF- κ B signalling may also involve in the regulation of neuroinflammation in AD. As shown in Figure 6, relevant protein levels were measured. We found that in cellular model, AD may activate the TLR4/MyD88/NF- κ B signalling. Meanwhile, milrinone treatment expectedly repressed the expression of this pathway. Some previous studies on AD inflammation suggested that the inhibition of TLR4/MyD88/NF- κ B signalling is crucial for the reduction of neuroinflammation in AD,^{42,43} which validated our experimental data that milrinone inhibited neuroinflammation through suppression of TLR4/MyD88/NF- κ B signalling pathway.

Conclusion

In a word, our in vivo experimental data revealed the neuroprotective role of milrinone on AD pathogenesis via the regulation of AB plaque enrichment, microglial activation, tau protein hyperphosphorylation, oxidative stress, and inflammation reactions. At the same time, the in vitro model results uncovered the inhibitory effect of

milrinone on the inflammatory-related TLR4/MyD88/NF- κ B signalling pathway. Our findings may provide a promising therapeutic drug for the amelioration of AD.

Data Sharing Statement

Raw data may be obtained from the corresponding author upon reasonable request.

Funding

This work supported by the Special fund for clinical research of Qiqihar Academy of Medical sciences(No. QMSI2020L-11).

Disclosure

The authors report no conflicts of interest in this work.

References

- Eratne D, Loi SM, Farrand S, Kelso W, Velakoulis D, Looi JC. Alzheimer's disease: clinical update on epidemiology, pathophysiology and diagnosis. *Australas Psychiatry*. 2018;26(4):347–357. doi:10.1177/1039856218762308
- Pottiez G, Yang L, Stewart T, et al. Mass-spectrometry-based method to quantify in parallel tau and amyloid beta 1–42 in CSF for the diagnosis of Alzheimer's disease. *J Proteome Res*. 2017;16(3):1228–1238. doi:10.1021/acs.jproteome.6b00829
- Katsouri L, Lim YM, Blondrath K, et al. PPARgamma-coactivator-1alpha gene transfer reduces neuronal loss and amyloid-beta generation by reducing beta-secretase in an Alzheimer's disease model. *Proc Natl Acad Sci U S A*. 2016;113(43):12292–12297. doi:10.1073/pnas.1606171113
- McGeer PL, McGeer EG. The amyloid cascade-inflammatory hypothesis of Alzheimer disease: implications for therapy. *Acta Neuropathol*. 2013;126(4):479–497. doi:10.1007/s00401-013-1177-7
- Gold M, El Khoury J. beta-amyloid, microglia, and the inflammasome in Alzheimer's disease. *Semin Immunopathol*. 2015;37(6):607–611. doi:10.1007/s00281-015-0518-0
- Nizami S, Hall-Roberts H, Warriar S, Cowley SA, Di Daniel E. Microglial inflammation and phagocytosis in Alzheimer's disease: potential therapeutic targets. *Br J Pharmacol*. 2019;176(18):3515–3532. doi:10.1111/bph.14618
- Ducatenzeiler P, Pichet-Binette A, Pimentel LSB. Neuronal driven pre-plaque inflammation in a transgenic rat model of Alzheimer's disease. *Neurobiol Aging*. 2014;35(10):2249–2262. doi:10.1016/j.neurobiolaging.2014.03.026
- Hoeijmakers L, Ruigrok SR, Amelanchik A, et al. Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model. *Brain Behav Immun*. 2017;63:160–175. doi:10.1016/j.bbi.2016.12.023
- Santos JRF, Bauer C, Schuchhardt J, et al. Validation of a prototype tau Thr231 phosphorylation CSF ELISA as a potential biomarker for Alzheimer's disease. *J Neural Transm (Vienna)*. 2019;126(3):339–348. doi:10.1007/s00702-019-01982-5
- Fujiwara H, Watanabe S, Iwata M, et al. Inhibition of microtubule assembly competent tubulin synthesis leads to accumulation of phosphorylated tau in neuronal cell bodies. *Biochem Biophys Res Commun*. 2020;521(3):779–785. doi:10.1016/j.bbrc.2019.10.191
- de Los Rios C, Marco-Contelles J. Tacrines for Alzheimer's disease therapy. III. The pyridotacrines. *Eur J Med Chem*. 2019;166:381–389. doi:10.1016/j.ejmech.2019.02.005

12. Birks JS, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst Rev.* 2018;6:CD001190. doi:10.1002/14651858.CD001190.pub3
13. Saito T, Hisahara S, Iwahara N, et al. Early administration of galantamine from preplaque phase suppresses oxidative stress and improves cognitive behavior in APPswe/PS1dE9 mouse model of Alzheimer's disease. *Free Radic Biol Med.* 2019;145:20–32. doi:10.1016/j.freeradbiomed.2019.09.014
14. McShane R, Westby MJ, Roberts E, et al. Memantine for dementia. *Cochrane Database Syst Rev.* 2019;3:CD003154.
15. Chou PS, Jhang KM, Huang LC, Wang WF, Yang YH. Skinfold thickness for rivastigmine patch application in Alzheimer's disease. *Psychopharmacology (Berl).* 2019;236(4):1255–1260. doi:10.1007/s00213-018-5135-x
16. Qasim A, Jain SK. Milrinone use in persistent pulmonary hypertension of the newborn. *Neoreviews.* 2020;21(3):e165–e178. doi:10.1542/neo.21-3-e165
17. Tang X, Liu P, Li R, et al. Milrinone for the treatment of acute heart failure after acute myocardial infarction: a systematic review and meta-analysis. *Basic Clin Pharmacol Toxicol.* 2015;117(3):186–194. doi:10.1111/bcpt.12385
18. Lannes M, Teitelbaum J, Del Pilar Cortes M, Cardoso M, Angle M. Milrinone and homeostasis to treat cerebral vasospasm associated with subarachnoid hemorrhage: the Montreal neurological hospital protocol. *Neurocrit Care.* 2012;16(3):354–362. doi:10.1007/s12028-012-9701-5
19. Labeyrie MA, Gaugain S, Boulouis G, et al. Distal balloon angioplasty of cerebral vasospasm decreases the risk of delayed cerebral infarction. *AJNR Am J Neuroradiol.* 2019;40(8):1342–1348. doi:10.3174/ajnr.A6124
20. Arac D, Erdi MF, Keskin F, et al. Neuroprotective effects of milrinone on experimental acute spinal cord injury: rat model. *World Neurosurg.* 2020. doi:10.1016/j.wneu.2020.12.021
21. Choi H, Huh J, Koo J, Lee J, Hwang W. Effects of milrinone on cerebral perfusion and postoperative cognitive function in spine surgery: secondary analysis of a CONSORT-compliant randomized controlled trial. *Medicine (Baltimore).* 2020;99(46):e21717. doi:10.1097/MD.00000000000021717
22. Saklani R, Jaggi A, Singh N. Pharmacological preconditioning by milrinone: memory preserving and neuroprotective effect in ischemia-reperfusion injury in mice. *Arch Pharm Res.* 2010;33(7):1049–1057. doi:10.1007/s12272-010-0711-6
23. Liu M, Chen F, Sha L, et al. (-)-Epigallocatechin-3-gallate ameliorates learning and memory deficits by adjusting the balance of TrkA/p75NTR signaling in APP/PS1 transgenic mice. *Mol Neurobiol.* 2014;49(3):1350–1363. doi:10.1007/s12035-013-8608-2
24. Sabogal-Guaqueta AM, Carrillo-Hormaza L, Osorio E, Cardona-Gomez GP. Effects of biflavonoids from *Garcinia madruno* on a triple transgenic mouse model of Alzheimer's disease. *Pharmacol Res.* 2018;129:128–138. doi:10.1016/j.phrs.2017.12.002
25. Milner MT, Maddugoda M, Gotz J, Burgener SS, Schroder K. The NLRP3 inflammasome triggers sterile neuroinflammation and Alzheimer's disease. *Curr Opin Immunol.* 2021;68:116–124. doi:10.1016/j.coi.2020.10.011
26. Xiao L, Zheng H, Li J, Wang Q, Sun H. Neuroinflammation mediated by NLRP3 inflammasome after intracerebral hemorrhage and potential therapeutic targets. *Mol Neurobiol.* 2020;57(12):5130–5149. doi:10.1007/s12035-020-02082-2
27. Gong Z, Huang J, Xu B, et al. Urolithin A attenuates memory impairment and neuroinflammation in APP/PS1 mice. *J Neuroinflammation.* 2019;16(1):62. doi:10.1186/s12974-019-1450-3
28. Chen Y, Qi Z, Qiao B, Lv Z, Hao Y, Li H. Oxymatrine can attenuate pathological deficits of Alzheimer's disease mice through regulation of neuroinflammation. *J Neuroimmunol.* 2019;334:576978. doi:10.1016/j.jneuroim.2019.576978
29. Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. *Sci Transl Med.* 2011;3(77):77sr71. doi:10.1126/scitranslmed.3002369
30. Takahashi RH, Nagao T, Gouras GK. Plaque formation and the intraneuronal accumulation of beta-amyloid in Alzheimer's disease. *Pathol Int.* 2017;67(4):185–193. doi:10.1111/pin.12520
31. Jin X, Liu MY, Zhang DF, et al. Baicalin mitigates cognitive impairment and protects neurons from microglia-mediated neuroinflammation via suppressing NLRP3 inflammasomes and TLR4/NF-kappaB signaling pathway. *CNS Neurosci Ther.* 2019;25(5):575–590. doi:10.1111/cns.13086
32. Jiang XW, Liu WW, Wu YT, et al. Notopterygium incisum extract (NRE) rescues cognitive deficits in APP/PS1 Alzheimer's disease mice by attenuating amyloid-beta, tau, and neuroinflammation pathology. *J Ethnopharmacol.* 2020;249:112433. doi:10.1016/j.jep.2019.112433
33. Walker D, Lue LF. Anti-inflammatory and immune therapy for Alzheimer's disease: current status and future directions. *Curr Neuropharmacol.* 2007;5(4):232–243. doi:10.2174/157015907782793667
34. Fink SL, Bergsbaken T, Cookson BT. Anthrax lethal toxin and Salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. *Proc Natl Acad Sci U S A.* 2008;105(11):4312–4317. doi:10.1073/pnas.0707370105
35. Heilig R, Dilucca M, Boucher D, et al. Caspase-1 cleaves bid to release mitochondrial SMAC and drive secondary necrosis in the absence of GSDMD. *Life Sci Alliance.* 2020;3(6):e202000735. doi:10.26508/lsa.202000735
36. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature.* 2015;526(7575):660–665. doi:10.1038/nature15514
37. Cheignon C, Faller P, Testemale D, Hureau C, Collin F. Metal-catalyzed oxidation of abeta and the resulting reorganization of Cu binding sites promote ROS production. *Metallomics.* 2016;8(10):1081–1089. doi:10.1039/C6MT00150E
38. Yao Y, Huang JZ, Chen Y, Hu HJ, Tang X, Li X. Effects and mechanism of amyloid beta1–42 on mitochondria in astrocytes. *Mol Med Rep.* 2018;17(5):6997–7004. doi:10.3892/mmr.2018.8761
39. Su Q, Li L, Sun Y, Yang H, Ye Z, Zhao J. Effects of the TLR4/Myd88/NF-kappaB signaling pathway on NLRP3 inflammasome in coronary microembolization-induced myocardial injury. *Cell Physiol Biochem.* 2018;47(4):1497–1508. doi:10.1159/000490866
40. Ju M, Liu B, He H, et al. MicroRNA-27a alleviates LPS-induced acute lung injury in mice via inhibiting inflammation and apoptosis through modulating TLR4/MyD88/NF-kappaB pathway. *Cell Cycle.* 2018;17(16):2001–2018. doi:10.1080/15384101.2018.1509635
41. Zhang Y, Zeng Y. Curcumin reduces inflammation in knee osteoarthritis rats through blocking TLR4/MyD88/NF-kappaB signal pathway. *Drug Dev Res.* 2019;80(3):353–359. doi:10.1002/ddr.21509
42. El-Sahar AE, Shiha NA, El Sayed NS, Ahmed LA. Alogliptin attenuates lipopolysaccharide-induced neuroinflammation in mice through modulation of TLR4/MYD88/NF-kappaB and miRNA-155/SOCS-1 signaling pathways. *Int J Neurosychopharmacol.* 2021;24(2):158–169. doi:10.1093/ijnp/pyaa078
43. Zhou J, Deng Y, Li F, Yin C, Shi J, Gong Q. Icariside II attenuates lipopolysaccharide-induced neuroinflammation through inhibiting TLR4/MyD88/NF-kappaB pathway in rats. *Biomed Pharmacother.* 2019;111:315–324. doi:10.1016/j.biopha.2018.10.201

Neuropsychiatric Disease and Treatment

Dovepress

Publish your work in this journal

Neuropsychiatric Disease and Treatment is an international, peer-reviewed journal of clinical therapeutics and pharmacology focusing on concise rapid reporting of clinical or pre-clinical studies on a range of neuropsychiatric and neurological disorders. This journal is indexed on PubMed Central, the 'PsycINFO' database and CAS, and

is the official journal of The International Neuropsychiatric Association (INA). The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/neuropsychiatric-disease-and-treatment-journal>