Rho Kinase Inhibitor Fasudil Protects against β -Amyloid-Induced Hippocampal Neurodegeneration in Rats

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Keywords

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SUMMARY

Background and purpose: Alzheimer's disease (AD) is a progressive neurodegenerative disorder, and $A\beta$ -induced neuronal damage is the major pathology of AD. There is increasing evidence that neuroinflammation induced by $A\beta$ is also involved in the pathogenesis of AD. Fasudil is a Rho kinase inhibitor and has been reported to have neuroprotective effects. In this study, the main purpose is to investigate whether fasudil has beneficial effects on cognitive impairment and neuronal toxicity induced by $A\beta$. **Methods and results:** In the present study, intracerebroventricular injection of $A\beta_{1-42}$ to rats resulted in marked cognitive impairment, severe neuronal damage, as well as increased IL-1 β , tumor necrosis factor alpha (TNF- α) production, and NF- κ B activation. Administration of fasudil significantly ameliorated the spatial learning and memory impairment, attenuated neuronal loss, and neuronal injury induced by $A\beta_{1-42}$. In addition, fasudil inhibited IL-1 β and TNF- α production and NF- κ B activation in the rat brain. **Conclusions:** Fasudil can protect against $A\beta$ -induced hippocampal neurodegeneration by suppressing inflammatory response, suggesting that fasudil might be a promising agent for the prevention and treatment of inflammation-related diseases, such as AD.

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The first two authors contributed equally to this work.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia in the elderly. Incidence of AD increases quickly, and AD has become the fourth cause of death in developed countries [1,2]. The hallmarks of AD brains are mainly characterized by senile plaques (SPs), which consist of aggregated β -amyloid protein ($A\beta$), and neurofibrillary tangles (NFTs) formed by hyperphosphorylated tau protein. Aggregated $A\beta$ induces neuronal damage in several different ways. It is toxic to neurons by causing mechanically destructive changes to the cell membrane [3]. Apart from the immediate toxicity of $A\beta$ on neurons, the aggregated $A\beta$ also activates microglia and astrocytes to produce proinflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) and interleukins (ILs) [4]. The proinflammatory cytokines induced by $A\beta$ could cause proapoptotic and synaptotoxic effects, which are extremely toxic to neurons [5]. Furthermore, there is evidence showing that inflammation might be a contributing factor to the formation of aggregated $A\beta$ and senile plaques, thus plays an important role in the

pathology of AD [6]. Therefore, inhibiting $A\beta$ -induced production of proinflammatory cytokine is an attractive strategy in the development of drugs for AD prevention and therapy.

There are many enzymes and proteins involved in neuroinflammation and neuropathology of AD, among which Rho kinase (ROCK) is an important one [7]. ROCK is one of the best-characterized downstream effectors of the small GTPases. Besides its typical effect on regulating actin cytoskeleton, ROCK is demonstrated to play a critical role in cell migration, chemotaxis, adhesion, reactive oxygen species formation, and apoptosis [8]. Furthermore, accumulating evidence suggests that ROCK-mediated pathway is associated with the process of inflammation [9], and inhibiting ROCK is beneficial to prevent neuroinflammation and further neuronal damage [10]. It was reported that nonsteroidal antiinflammatory drugs could reduce the incidence of AD by inhibiting the activity of ROCK [11]. It was also found that ROCK inhibitor improved cognitive function of rats [12]. Therefore, ROCK might be a promising target for AD treatment.

Fasudil, a potent ROCK inhibitor, is widely used to treat cerebral vasospasms occurring after subarachnoid hemorrhage [13].

Studies have reported that fasudil protects neurons against injury after cerebral ischemia and hypoxia–reoxygenation in animal models [11,14]. In humans, treatment with fasudil within 48 h of onset of acute ischemic stroke significantly improved the patients' clinical outcome [15]. These findings indicated that fasudil was a promising neuroprotective agent. Fasudil also showed antiinflammatory effect, which suppressed inflammatory response in autoimmune encephalomyelitis by inhibiting T-cell proliferation and infiltration [16].

In the present study, we carried out a set of *in vivo* experiments to verify whether fasudil has neuroprotective effect in a rat model of AD induced by A β and investigate the possible mechanisms. We found that fasudil had beneficial effects on memory impairment, neuronal loss, and neuronal injury in AD model rats. The mechanic study showed that fasudil suppressed neuroinflammatory response through reducing inflammatory factor production and inhibiting NF- κ B activation.

Materials and Methods

Reagents

Fasudil (30 mg; 2 mL/ampoule) was produced by Tianjin Chase Sun Pharmaceutical Co., Ltd. (Tianjin, China). A β_{1-42} was purchased from Sigma (St. Louis, MO, USA). IL-1 β and TNF- α ELISA kits were obtained from Boster (Wuhan, China). TUNEL Staining Kit was manufactured by Roche (Indianapolis, IN, USA). Rabbit anti-rat NF- κ B p65 polyclonal antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Animal Models

Male Wistar rats, weighting 260–300 g, were supplied by the animal laboratory of Shandong University of Traditional Chinese Medicine (Jinan, China). They were maintained in a constant temperature ($22.5 \pm 2.5^\circ\text{C}$) and humidity ($60 \pm 10\%$) environment under a 12-h light/dark cycle and were allowed food and water *ad libitum*. The rats were randomly divided into control group, model group (A β_{1-42}), fasudil 5 mg/kg group (A β_{1-42} +fasudil 5 mg/kg), and fasudil 10 mg/kg group (A β_{1-42} +fasudil 10 mg/kg). There are equally 20 rats in each group.

A β_{1-42} was dissolved in normal saline at the concentration of 5 $\mu\text{g}/\mu\text{L}$ and incubated at 37°C for 72 h to allow the peptide to aggregate. The rats were anesthetized and then placed in a stereotaxic apparatus (Tianjin Institute of Medical Devices, Tianjin, China). An injection cannula was inserted into the left lateral ventricle (AP: -0.8 mm from bregma; ML: 1.3 mm from midline; DV: 3.6 mm from the dura). After the skull was opened, incubated A β_{1-42} (10 $\mu\text{g}/2 \mu\text{L}$) or normal saline as control was injected within 5 min through the guide cannulas. The needle was left in place for another 10 min before it was slowly withdrawn.

Drug Treatment

From the first day after surgery, rats were administrated with fasudil (5 mg/kg and 10 mg/kg, *i.p.*) once a day for consecutive 14 days. The dose of fasudil was selected based on the previous

behavioral tests. The control and model group received the same volume of normal saline as that of fasudil.

Morris Water Maze Test

The apparatus of Morris Water Maze (Chinese Academy of Medical Sciences, Beijing, China) is a circular water tank (150 cm in diameter and 50 cm high) filled with water maintained at $24 \pm 0.5^\circ\text{C}$. The water was made opaque by adding milk. A platform (10 cm in diameter) was placed at the midpoint of one quadrant and submerged 1 cm under the water surface. The sessions were monitored by a digital camera linked to a computer through an image analyzer. On the 9th day after A β injection, two training trials each day were conducted for five consecutive days while administration was continued during the trials. For each trial, the rats ($n = 10$ in each group) were placed in the pool at one starting position and were given 120 second to find the escape platform. If a rat failed to reach the platform within 120 second, it was guided to the platform by the experimenter, and the time was recorded as 120 second. The average escape latency and swimming velocity of each rat in the two trials were counted as the individual result of a rat per day. Meanwhile, the swimming path was monitored. One day after the training period, rats were tested on a spatial probe trial in which the platform was removed, and they were allowed to swim freely for 120 second. The time spent in the target quadrant and the number of crossing the platform were recorded.

Pathological Tissue Processing

After the behavioral experiments, the rats ($n = 10$ in each group) were killed via intraperitoneal injection with 10% chloral hydrate (4 mL/kg) and perfused transaortically first with 0.9% sodium chloride followed by 4% paraformaldehyde (pH 7.4, 4°C). Then, the rats were decapitated and the hippocampus segments were dissected and postfixed in the same 4% paraformaldehyde solution at 4°C for 48 h, after which they were rinsed, dehydrated with alcohol, pellucidumed with xylene, and embedded in paraffin. Coronal sections (4 μm) were successively cut for HE staining, Nissl staining, and TUNEL assay.

One hippocampus segment in each group was selected randomly for electron microscopic section. The segments were divided into $1 \times 1 \times 1$ mm slices in CA1 region and were postfixed in 2.5% glutaraldehyde solution at 4°C for 24 h, thereafter in osmium tetroxide phosphate buffer at 4°C for 2 h. Then, they were rinsed, dehydrated with acetone, embedded in epoxy resins, and sliced by ultra-thin slicer.

HE Staining

The mounted sections were submerged in hematoxylin staining solution for 15 min and rinsed in distilled water for 5 min at room temperature, and then, they were dipped into hydrochloric acid alcohol for 15 second. After the sections were rinsed three times, they were stained by eosin staining solution for 30 min at room temperature. Later, the sections were rinsed again and dehydrated in graded series of ethanol, immersed in xylene, and coverslipped with mounting medium.

Electron Microscopy

The ultra-thin slices were loaded on a Formvar/Carbon-coated grid and double stained by uranyl acetate and lead citrate. Thereafter, they were observed and filmed under JEM-1200EX transmission electron microscope (Japanese electronics company, Tokyo, Japan).

Nissl Staining

The brain sections were Nissl-stained with toluidine blue for neuronal cell bodies. The brain sections were then mounted, air-dried, dehydrated, and coverslipped. The prepared sections were observed by light microscopy (NIKON E600, Tokyo, Japan) and analyzed using the Image-Pro plus system.

TUNEL Assay

After deparaffinization and rehydration, the brain sections were digested in proteinase K (20 mg/mL). Then, the tissue was rinsed in PBS three times for 5 min and treated with 0.25 mg/mL bovine serum albumin. Sections were incubated at 37°C for 60 min with labeling solution containing 4 μ L TdT, 1 μ L fluorescein isothiocyanate (FITC)-16-dUTP, and 45 μ L equilibrium liquid. After washing with PBS, the sections were blocked with antifluorescence quenching liquid. Then, they were observed with fluorescence microscopy, and the TUNEL-positive cells were expressed as the number of green fluorescent.

Cytokine Assay by ELISA

Remnant animals in each group were decapitated. The brains were immediately taken out and the hippocampus tissues were isolated. Then, they were dissected, snap frozen on dry ice, homogenized, and diluted in the provided buffer containing protease inhibitortease. IL-1 β and TNF- α were measured by sandwich ELISA kits following the manufacturer's instructions. Determinations were performed in duplicate, and the results were expressed as pg/mL.

Western Blot Assay

Rat hippocampus was lysed in nondenaturing lysis buffer. The lysates were then centrifuged at 12,000 *g* for 15 min at 4°C; the supernatants were mixed with loading buffer and boiled for 5 min. Protein concentration was measured by Bradford protein assay. Samples containing 30 μ g of protein per lane were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes. The membranes were blocked in 5% skim milk TBST (20 mmol/L Tris-HCl, pH 7.5, 500 mmol/L NaCl, 0.1% Tween 20) for 1 h, then NF- κ B p65 antibody was added in the same milk and incubated overnight at 4°C, and then incubated with horseradish peroxidase-conjugated secondary antibody in TBST for 2 h at room temperature. The blot was developed with LAS3000 chemiluminescence system (Fujifilm, Tokyo, Japan), and the densities of the bands were determined using Gel-Pro Analyzer 4.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical Analysis

The SPSS 17.0 (IBM Corp., New York, NY, USA) was used for all calculations and statistical evaluations. The data from training trial in the Morris water maze were analyzed by two-way ANOVA followed by LSD test. Other differences were tested by one-way ANOVA with LSD test. Results were presented as means $\pm$ SD, and a level of $P < 0.05$ was considered significant.

Results

Fasudil Reduced Learning and Memory Deficits of Rats Injected with A β ₁₋₄₂

To explore whether fasudil could improve cognitive impairment in A β ₁₋₄₂-induced AD rat model, Morris water maze test was performed. The escape latency was used to reflect an aspect of spatial learning. Two-way ANOVA revealed a significant treatment effect on escape latency among groups ($F_{3,36} = 21.85$, $P < 0.01$). Also, the results showed a significant day effect on escape latency within groups ($F_{4,144} = 56.42$, $P < 0.01$), indicating that all groups of rats improved their performance over the 5-day training period. However, the interaction treatment $\times$ trial did not affect the parameter significantly ($F_{12,144} = 1.71$, $P > 0.05$). *Post hoc* analysis confirmed the internal validity of the study. The latency of A β ₁₋₄₂-injected rats was much longer than that of control rats on day 2, 4, and 5. Fasudil 10 mg/kg significantly decreased the latency of rats to find the platform on day 3 and 5 ($P < 0.01$, Figure 1A). We also observed that there were no differences of swimming velocity among the four groups over the 5-day training period (Figure 1B). As for swimming path, after a 5-day training, the linear and tendency mode increased while the random and marginal mode decreased, especially in the control and fasudil 10 mg/kg treated rats (Figure 1C).

On the probe trial day when the platform was moved, rats were expected to spend the majority of the trial searching for the platform in the target quadrant to detect the memory ability. Rats treated with fasudil 10 mg/kg spent significantly more time in the correct quadrant than the A β ₁₋₄₂-injected rats ($P < 0.01$; Figure 1D). The number of A β ₁₋₄₂-injected rats crossing the platform remarkably decreased compared with the normal rats, while fasudil treatment significantly improved the number of crossing the platform ($P < 0.01$; Figure 1E). These results suggested that fasudil had beneficial effects on learning and memory dysfunction of rats impaired by A β ₁₋₄₂.

Fasudil Attenuated Neuronal Injury in the Hippocampus of Rats Injected with A β ₁₋₄₂

Through HE staining, we observed that neurons in the hippocampus of rats in control group were oval shaped, tightly packed, evenly stained, and the nuclei were round and clear, while neurons in the hippocampus of A β ₁₋₄₂-injected rats were sparsely arranged, and the nuclei were deeply stained as well as condensed. The neuronal loss and neuronal injury we observed on the A β ₁₋₄₂-injected rats were consistent with other reports [15]. Compared with the A β ₁₋₄₂-injected rats, neurons in the hippocampus of fasudil 10 mg/kg treated rats were arranged neatly in

Figure 1 Fasudil treatment attenuated the learning and memory deficits of rats intracerebroventricularly injected with A β_{1-42} . The Morris water maze test training began on the 9th day after A β_{1-42} injection. Two trials each day were conducted for 5 days. Rats were i.p. injected with fasudil 5 mg/kg and 10 mg/kg for 14 days. **(A)** The escape latencies during the training trial sessions. **(B)** The swimming velocity during the training trial sessions. **(C)** Representative swimming paths. **(D)** The time spent in the target in the spatial probe test. **(E)** The number of crossing the platform in the spatial probe test. Values were expressed as means $\pm$ SD, $n = 10$ in each group. ** $P < 0.01$ versus control rats; ## $P < 0.01$ versus A β_{1-42} -injected rats.

rows, oval or round, rarely depigmented, and the nuclei were clearly visible (Figure 2A). The morphological change induced by fasudil is a clear indication of its neuroprotective effects.

We further observed the ultrastructure of neurons in the hippocampus of rats. The electron microscopy results showed that the structures of most neurons of rats in the control group were normal, which mainly contained euchromatin nucleus and round nucleus. These cells were also rich in organelles. Neurotoxicity showed by neuron recession, cell shrinkage, membrane bump, reduced organelles, and structural damage were observed on the A β_{1-42} -injected rats. For the fasudil 10 mg/kg treated rats, there

were fewer above-mentioned pathological changes (Figure 2B). These results indicated that fasudil could attenuate A β_{1-42} -induced neuronal loss and injury in the hippocampus of rats.

Nissl staining of neuronal cell bodies was also performed to evaluate the neuroprotective effect of fasudil. Compared with rats in the control group, the number of Nissl-positive cells greatly decreased in the hippocampus ($P < 0.01$) of A β_{1-42} -treated rats, which indicated the neurotoxicity of A β . Fasudil 10 mg/kg significantly increased the number of Nissl-positive cells ($P < 0.05$; Figure 2C,D). These results verified that fasudil attenuated the dysfunction of neuronal cells.

Figure 2 Fasudil attenuated neuronal injury in the hippocampus of rats injected with A β_{1-42} . **(A)** HE staining in the hippocampus of rats (magnification 40 $\times$ and 400 $\times$). **(B)** Ultrastructural hippocampus of rats observed under electron microscope. **(C)** Histochemical staining of Nissl in the hippocampus of rats in each group (magnification 40 $\times$ and 400 $\times$). **(D)** The quantitative analysis of Nissl staining. Values were expressed as means $\pm$ SD from three independent experiments in each animal, $n = 6$ in each group. ** $P < 0.01$ versus control rats; # $P < 0.05$ versus A β_{1-42} -injected rats.

Fasudil Inhibited Neuronal Apoptosis in the Hippocampus of Rats Injected with A β_{1-42}

The TUNEL-positive neurons showed chromatin condensation in the perinuclear regions, which are generally regarded as hallmarks of apoptosis. As shown in Figure 3, almost no TUNEL-positive neurons were detected in the hippocampus of rats in the control group, while extensive TUNEL-positive neuronal staining appeared in the A β_{1-42} -injected rats ($P < 0.01$). There were much fewer TUNEL-positive cells in the fasudil-treated rats compared with the ones in the A β_{1-42} -injected rats (10 mg/kg: $P < 0.01$; 5 mg/kg: $P < 0.05$; Figure 3). These findings demonstrated that fasudil protected neurons against apoptosis.

Fasudil Inhibited IL-1 β and TNF- α Production in the Hippocampus of Rats Injected With A β_{1-42}

Neuroinflammation is characterized by increasing proinflammatory cytokine levels, such as IL-1 β and TNF- α . In this study, the lev-

els of IL-1 β and TNF- α in brain tissue lysate were measured using ELISA assay. As shown in Figure 4, the levels of IL-1 β and TNF- α significantly increased in the hippocampus of rats injected with A β_{1-42} ($P < 0.01$). Fasudil 5 mg/kg and 10 mg/kg significantly decreased the levels of IL-1 β (5 mg/kg: $P < 0.05$; 10 mg/kg: $P < 0.01$) and TNF- α (5 mg/kg: $P < 0.05$; 10 mg/kg: $P < 0.01$) in dose-dependent manners, suggesting that fasudil suppressed inflammatory response in the rat brain (Figure 4).

Fasudil Inhibited NF- κ B Activation in the A β_{1-42} -treated Rat Hippocampus

NF- κ B is responsible for regulating the expression of a wide variety of genes. The most abundant form of the protein is a heterodimer of p50 and p65 subunits, in which the p65 subunit contains the transcriptional activation domain. Western blot assay showed that the levels of NF- κ B p65 in cell nucleus of hippocampus significantly increased after intracerebroventricular injection of A β_{1-42} ($P < 0.01$). Fasudil 10 mg/kg markedly reduced the level of NF-

Figure 3 Fasudil inhibited neuronal apoptosis in the hippocampus of rats injected with A β_{1-42} . (A) Representative photomicrographs of TUNEL labeling in the hippocampus. (B) The quantitative analysis of TUNEL-positive cells. Values were expressed as means $\pm$ SD from three independent experiments in each animal, $n = 6$ in each group. ** $P < 0.01$ versus control rats; # $P < 0.05$ and ## $P < 0.01$ versus A β_{1-42} -injected rats.

Figure 4 Fasudil inhibited IL-1 β and tumor necrosis factor alpha (TNF- α) production in the hippocampus of rats injected with A β_{1-42} . (A) ELISA assay of IL-1 β concentration in the hippocampus of rats. (B) ELISA assay of TNF- α concentration in the hippocampus of rats, $n = 10$ in each group. ** $P < 0.01$ versus control rats; # $P < 0.05$ and ## $P < 0.01$ versus A β_{1-42} -injected rats.

κ B p65 in nucleus ($P < 0.01$; Figure 5), which indicated the inhibiting effect of fasudil on NF- κ B activation.

Discussion

In the present study, it was demonstrated that intracerebroventricular injection of A β_{1-42} induced significant inflammatory response, neuronal death in the hippocampus, and memory deficits in the rats. Accordingly, we firstly found that fasudil remarkably ameliorated the spatial learning and memory impairment, attenuated neuronal loss, and neuronal injury induced by A β_{1-42} *in vivo*. Furthermore, our data showed that fasudil inhibited IL-1 β and TNF- α production as well as NF- κ B activation, indicating that fasudil might also suppress inflammatory response in the brain.

It has been reported that ROCK inhibitors have neuroprotective effects. Y-27632 is a potent ROCK inhibitor, and it was reported to protect retinal ganglion cells from serum deprivation or growth factor deprivation and axotomy-induced apoptosis [17]. Studies also showed that ROCK inhibitor could rescue cells from apoptosis induced by ischemia injury [18]. Another research reported that ROCK inhibitor increased dendrite branching and promoted lengthening of the dendrite arbors of CA1 pyramidal neurons [19]. Furthermore, a recent study indicated that nonsteroidal anti-inflammatory drugs (NSAIDs) reduced A β production by inhibiting the RhoA-ROCK pathway [20]. In addition, there is some evidence suggested that inhibition of ROCK could promote neurite outgrowth and reduce A β secretion induced by Nogo-P4 of the cultured cortical neurons [21]. All these studies have demonstrated the involvement of the ROCK pathway in AD. However,

the effects of ROCK and its inhibitors on A β -associated models *in vivo* have been indicated unclear. Fasudil is a well-known ROCK inhibitor, and our present study provided the first evidence that fasudil attenuated hippocampal neuronal damage and improved cognitive dysfunction in A β -induced AD rat model. The results were consistent with previous studies, which showed that fasudil improved learning and memory ability in aged rats [12] and had neuroprotective effects on cerebral ischemia-induced neuronal injury in animal models [14]. Our findings gave a hint that ROCK inhibitor, such as fasudil, might be beneficial to treat diseases related with A β -induced neurotoxicity, such as AD.

Apart from that, ROCK inhibitors were also reported to suppress the proliferation and activation of microglia [22,23] and accordingly reduce the production of inflammatory cytokines such as IL-1 β , TNF- α , and IFN- γ [24]. The work of Ding et al. [11] reported that fasudil protected neurons against hypoxia-reoxygenation injury by suppressing microglial inflammatory response in the hippocampus. Therefore, we investigated the effect of fasudil on neuroinflammation. Our present data demonstrated that after the rats were intracerebroventricularly injected with A β_{1-42} , the levels of inflammatory cytokines, such as IL-1 β and TNF- α , significantly increased in the hippocampus. The results indicated that the inflammatory response was induced by A β , and due to the well-known toxicities of the inflammatory factors, the inflammatory effect might contribute to the neurotoxicity. Fasudil treatment markedly attenuated the inflammatory response, suggesting that the neuroprotection of fasudil may be related to its antiinflammatory effects.

NF- κ B is present in neuronal and glial cells, which is a transcription factor that involved in proinflammatory gene activation as well as other genes regulations. Most target genes regulated by NF- κ B are related to immune and inflammatory response, such as genes of proinflammatory cytokines like IL-1 and TNF- α , and genes of enzymes like inducible nitric oxide synthase (iNOS) and COX-2 [25]. It was well documented that blockage of NF- κ B transcriptional activity in the microglial nucleus could suppress proinflammatory cytokine production [26]. In the present study, we found that A β challenge resulted in translocation of the NF- κ B p65 subunit to the nucleus, which could cause the activation of NF- κ B and its target genes. Fasudil remarkably inhibited NF- κ B p65 translocation, indicating that the antiinflammatory effect of fasudil may be through inhibiting NF- κ B activation.

Conclusively, we evaluated fasudil's beneficial effects on the inhibition of A β_{1-42} -induced neuronal injury, memory impairment, and neuroinflammation in rats for the first time. Due to the fact that neuroinflammation is closely associated with the neuronal injury, we speculated that the neuroprotective effects of fasudil might be through suppressing the neuroinflammatory response. Although further experiment is needed to clarify the relationship between antiinflammatory effect and neuroprotective effect of fasudil, the dramatic benefit of fasudil in A β_{1-42} -induced animal models makes it a promising strategy for the prevention and treatment of inflammation-related diseases, such as AD.

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Figure 5 Fasudil inhibited NF- κ B activation in the hippocampus of rats injected with A β_{1-42} . **(A)** Western blot analysis of NF- κ B p65 in the nucleus extract in each group. **(B)** The relative optical density was normalized to histone. Values were expressed as means $\pm$ SD, $n = 10$ in each group. ** $P < 0.01$ versus control rats; ## $P < 0.01$ versus A β_{1-42} -injected rats.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Data S1. Rho kinase (ROCK) activity assay.

Figure S1. Effect of fasudil on Rho kinase (ROCK) activity in the hippocampus of rats.