Allicin can reduce neuronal death and ameliorate the spatial memory impairment in Alzheimer’s disease models


ABSTRACT

Objectives: To investigate the mechanisms and protective effects of allicin on learning and memory in a mouse model of Alzheimer’s disease (AD).

Methods: This study took place in the Institute of Medicine of Jishou University, Jishou, Hunan Province, China, between January and September 2009. Allicin was given as preventive administration after AD was induced by amyloid beta (Aβ(1-42)), and the protective effects of Allicin against learning and memory impairment were investigated. Sixty mice were randomly divided into 3 groups including the sham-operated+phosphate buffer solution (PBS) group, the Aβ(1-42)+PBS group, and the Aβ(1-42)+allicin group. The Aβ(1-42) (1 µL = 4 µg) was injected into the bilateral hippocampi. Sham-operated mice were infused with PBS. Allicin or PBS was then injected intraperitoneally for 14 days. The animals were trained, and learning and memory abilities tested using the Morris Water-Maze. The changes of Aß(1-42) and P38 mitogen-activated protein kinase (p38MAPK) were recorded to explore the mechanism of allicin’s protective effects on learning and memory deficits.

Results: The Aß(1-42)-infused allicin-treated group showed significantly shorter latency times than the PBS treated Aß(1-42)-infused group from the second day of learning sessions (p=0.031), accompanied with significant reduction of malondialdehyde (MDA) (p=0.035) and an increase of superoxide dismutase (SOD) activity (p=0.041). Allicin also decreased Aß and p38MAPK expressions in the cerebral cortex of AD mice model (p=0.031).

Conclusion: Preventive administration of allicin prevented learning and memory impairment, the mechanism may be due to an increase in the activity of SOD, a reduction in the levels of MDA and the expressions of Aß and p38MAPK in the brain.


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Alzheimers disease (AD) is a progressive neurological disorder characterized by loss of memory cognition. Oxidative stress is a key factor in the pathogenesis of AD, and there is strong evidence of free radical oxidative damage, particularly of neuronal lipids, proteins, nucleic acids, and sugars, occurring in AD brains. Recently, it has been reported that RNA and protein oxidation and lipid peroxidation are also significantly elevated in vulnerable regions of the mild cognitive impairment (MCI) brain, suggesting oxidative damage may be an early event in the pathogenesis of AD. Allicin is the most important lipid-soluble chain breaking natural antioxidant in mammalian cells, and can cross the blood-brain barrier and accumulate at therapeutic levels in the brain, where it reduces lipid peroxidation. In recent years, allicin has been reported to have neuroprotective effects in various experimental neurodegenerative disease models, such as cerebral ischemia, acute brain infarction, and amyotrophic lateral sclerosis. At present, only a few studies are focused on the therapeutical potential of allicin in vascular dementia, where it decreases the loss of neural cells in the hippocampus and cortex of temporal and frontal lobes and improves the learning and memory abilities of the rats. The data suggest that the antioxidant actions of allicin may have an important role in the antiaging effects. The potent antioxidant action is one of the mechanisms of allicin. Allicin may prevent the formation of lipid peroxides, protect proteins and DNA from oxidative damage, and decrease inflammation. The studies provide a theoretical basis for allicin treating AD. According to reports, in our study, allicin was given as preventive administration after an AD model was induced, and the protective effects of allicin against learning and memory impairment were investigated. For the testing of putative, cognition-enhancing agents, the establishment and standardization of animal cognitive deficit models are required. This study was designed to evaluate the protective effect of allicin on the learning and memory impairment in an AD model induced by Aβ(1-42).

**Methods.** This study was carried out in the Institute of Medicine of Jishou University, Jishou, China, between January and September 2009. Allicin was given as preventive administration after an AD model was induced by Aβ(1-42), and the protective effects of allicin against learning and memory impairment were investigated. Allicin was obtained from the Chia Tai Group, Tianjin, China (Lot 060111, 60mg/5ml), and the Aβ(1-42) from US Peptide (Sigma, St. Louis, MO, USA). Anti-β-Actin, anti-p38, anti-Aβ(1-42) antibodies were obtained from Cell Signaling (Sigma, St. Louis, MO, USA). Male Kunming mice (18-22 g from the animal facility of the Jishou University) were housed 10 per cage with free access to food and water, and were kept in a constant environment (22±2°C, 50±5% humidity, 12-hour light/dark cycle). Sixty mice were randomly divided into 3 groups, including the sham-operated+PBS group, the Aβ(1-42)+PBS group, and the Aβ(1-42)+allicin group. The Aβ(1-42) was dissolved in sterile distilled water at a concentration of 4µg/µL, and incubated at 37°C for 7 days to obtain the aggregated form. Under anesthetization, peptides (1 µL=4µg) were injected into the bilateral hippocampi, with stereotaxic coordinates from the bregma in mm, A-3,L/R-2.0, and V 3.5. Sham-operated mice were infused with PBS.

Allicin or PBS was then injected intraperitoneally (i.p) for 14 days. The allicin concentration used in this study (180 mg/kg/day) was chosen, based on the report by others. All experimental animals were overseen and approved by the Animal Care and Welfare Committee of Jishou University before and during the experiments. The animals were then trained, and learning and memory abilities were tested using the Morris Water-Maze. White-colored water was poured into a circular pool (diameter - 73 cm; height - 42 cm), and a white platform (diameter: 8.3 cm) was placed 1.5 cm below the water level in the middle of a fixed quadrant. The temperature of the water was kept constant throughout the experiment (24±1°C). Animals were required to find a submerged platform (8.5 cm in diameter, 35 cm high) in the pool using the spatial cues. The 2 starting points were changed daily. Animals were trained for 5 days, the latency to escape on to the hidden platform was recorded. After the final training session, a single probe trial was conducted. The escape platform was removed, and each mouse was allowed to swim for 120 s in the maze. The number of times the mice crossed the annulus where the platform had been located was recorded. At the end of the behavioral observation, all the mice were sacrificed. The right brain was put onto an ice plate, and prepared to 10% tissue homogenate with 0.9% saline, and centrifuged at 3000 revolution for 10 minutes. The superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in the brain of the mice were measured using a kit (Jiancheng Institute of Biotechnology, Nanjing, China) according to the manufacturer's directions.

The mice were anesthetized with 10% chloral hydrate (24.3g/kg), i.p. Then the mice were perfused with 100 ml 0.9% sodium chloride solution and subsequently with 4% paraformaldehyde in 0.1 mol/l...
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PBS at 7.4 pH. The left brains were removed and post-fixed for 24 hours in the same fixative. The post-fixed brains were cryo-protected in 25% sucrose in PBS. Then the brains were removed, paraffin-embedded, and coronally sectioned at 6 mm thickness. The sections were stained with hematoxylin and eosin (H & E). Then the neuronal damage was assessed under a microscope, and was expressed as a percentage of the number of eosinophilic cells/the total number of cells in each region in the hippocampal area.

Fixed lefts brains in 10% neutral buffered formalin for 48 hours were dehydrated and embedded in paraffin. After dehydration through graded alcohols to water, a primary antibody was revealed by incubating the cells for 45 minutes with CyTM3-conjugated secondary antibody (Molecular Probes, Carlsbad, CA, USA). After 3 washes with permeabilization buffer and one wash with PBS, cells were mounted on microscope slides in mounting medium (DAKO, CA, USA). Confocal microscopic observation was performed using an Olympus FV300 (Olympus, Tokyo Japan). The right hippocampus were excised and immediately frozen to -80°C for analyses of Aβ, and p38MAPK. Protein was resolved in sodium dodecyl sulfate polyacrylamide gel, electrophoresed at 30-50 mg of protein/lane, and transferred onto a nitrocellulose membrane (Amersham Pharmacia, Buckinghamshire, UK). The bands were probed with p38MAPK (1:5000), Aβ(1-42) (1:2000) and detected using horseradish peroxidase-conjugated secondary antibody (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA). Immunoreactive bands were visualized using an enhanced chemiluminescence system (ECL; Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA) according to the manufacturer’s instructions. The protein bands were quantitatively analyzed by Image-Pro software (Eastman Kodak Company, New Haven, CT, USA), and the amount of protein was expressed as relative level of sum optical density.

All results were shown as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by the least significant difference method (LSD) was adopted for multiple group comparison. Data was analyzed using the Statistical Program for Social Sciences (SPSS Inc, Chicago, IL, USA) statistical program.

**Results.** Herein, we assessed the effects of allicin in an in vivo AD model. Two weeks after completing the intraperitoneal injections, we tested spatial learning and memory impairment using the Morris Water-Maze test in the PBS- or allicin treated animals of the Aβ(1-42) or sham-operated groups. The Aβ(1-42)-infused allicin-treated group showed shorter latency times than the PBS-treated Aβ(1-42) infused group from the second day of these learning sessions (*p*=0.031, Figure 1a). We confirmed no noticeable differences between the allicin-treated animals or sham-operated groups (Figure 1a). To confirm whether the memory impairment shown in the Aβ(1-42) infused mice were actually attenuated by allicin treatment, we performed the probe test, and recorded the average latency during the stay at zone one without the platform. The allicin-treated mice stayed significantly longer (32.89 ±
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After the allicin treatment (180 mg/kg) for 14 days, we checked neuronal cell death by H & E staining in the 3 groups mice, and found that allicin treatment significantly reduced neuronal death in the hippocampus and dentate gyrus (Figure 2a). The percentage of eosinophilic cells versus total cells in the dentate gyrus was 8.4% for the sham-operated groups, and 48.6% for the Aβ(1-42) infused groups, and 18.3% in the allicin-treated Aβ(1-42) infused mice. In the CA3, the percentage of eosinophilic cells versus total cells was 19.3% for the sham-operated groups, and 60.7% for the Aβ(1-42) infused groups, and 30.4% in the allicin-treated Aβ(1-42) infused mice (Figure 2b). The results of the SOD activity and MDA content are shown in Figure 3. Figure 3a shows the content of MDA in the brain, and increased from 47.81 ± 17.6 nmol/mg protein in the sham-operated groups to 71.08 ± 11.69 nmol/mg protein in the Aβ(1-42) injection group. In the allicin group, the MDA content also decreased from 71.08 ± 11.69 nmol/mg protein in the Aβ(1-42) injection group to 58.34 ± 14.14 nmol/mg protein (p=0.036), indicating that allicin was effectively inhibiting the production of MDA in the AD brain model. In the experiment, SOD activity decreased in the Aβ(1-42) infused compared with the sham-operated groups from 72.3 ± 17.4 U/mg protein to 42.6 ± 14.8 U/mg protein (p=0.037), and in the allicin group, the SOD activity was elevated from 42.6 ± 14.8 U/mg protein to 65.8 ± 16.3 (p=0.043) as shown in Figure 3b.

The effects of allicin on the expression of p38MAPK and Aβ were investigated by immunohistochemistry.
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Figure 4 - Immunohistochemical evaluation of the effects of allicin on the expression of p38 mitogen-activated protein kinase (p38MAPK) and amyloid beta (Aβ[1-42]) (n=5, original magnification x200) showing: a) the expression of Aβ(1-42) in the hippocampus and dentate gyrus, b) The expression of p38MAPK in hippocampus and dentate gyrus. The fluorescent immunohistochemistry was performed in CA1 and CA3 and dentate gyrus with p38MAPK or Aβ(1-42) antibody overnight and visualized using Cy3-conjugated secondary antibody. Images were collected using the Image-Pro program on an Olympus FV300 (Olympus, Tokyo, Japan). Scale bar indicates 100 mm; inset also 100 mm. The results are representative of 5 separate experiments performed with different samples.

Figure 5 - Western blotting evaluation of the effects of allicin on the expression of p38 mitogen-activated protein kinase (p38MAPK) and amyloid beta (Aβ[1-42]) showing: a) the expression of Aβ(1-42) in the brain of the 3 groups, b) The expression of p38MAPK in the brain of the 3 groups. c & d) The results were expressed as the mean ± SD (n=4); **p=0.003 versus sham + phosphate buffer solution (PBS), *p=0.031 versus Aβ(1-42)+PBS.

after the allicin treatment (180 mg/kg) for 14 days. To examine the in situ distribution of p38MAPK and Aβ, sections from the hippocampus of control and Aβ(1-42) infused mice were stained with p38MAPK or Aβ-specific antibody. The immunoreactivities of p38MAPK and Aβ in the CA1 and CA3 of the hippocampus and that dentate gyrus of Aβ(1-42)-infused mice were found to be increased significantly compared to the sham-operated groups (Figure 4) (data not shown). Intraperitoneal allicin for 2 weeks, after Aβ(1-42) having been infused into mice lateral ventricles continuously for a week significantly reduced
the p38MAPK immunoreactivities (Figure 4) (data not shown). The Aβ is reported to increase p38MAPK activation in neuronal cells. We investigated the effects of allicin on p38MAPK activation caused by Aβ(1-42). Results from Western blots showed that allicin attenuated activation of p38MAPK induced by Aβ(1-42) treatment (Figure 5).

**Discussion.** The p38MAPK plays a key role in the regulation of inflammatory cytokine production and is involved in many inflammatory processes. It is widely reported that inflammation in the CNS is part of the pathogenesis of AD. Moreover, the p38MAPK microglial signal transduction pathway plays the pivotal role in the inflammatory response to Aβ(1-42) deposit in vivo. The p38MAPK was significantly activated in microglia, astrocytes and neurons, around and distant from the plaques in neuregenerative diseases (TgCRND8) mice expressing a double mutant form of human amyloid precursor protein, and representing a good model of Alzheimer's disease. The activation of p38MAPK by Aβ peptide may provide some clues in this area of AD research. Increased p38MAPK activity will lead to overproduction of a variety of inflammatory cytokines, which in turn, trigger inflammatory responses and mediate gliosis, a common histopathologic observation in the brains of patients with AD. Activation of the p38MAPK-MAPKAPK signaling pathway is responsible for the induction of actin stress fibers induced by overproduction of Aβ peptide. Meanwhile, p38MAPK inhibition prevents Aβ(1-42)-mediated down-regulation of occludin. Amyloidβ25-35 can activate p38MAPK signal transduction pathways and lead to the increased expression of p38MAPK in the olfactory bulb of rats with AD. The p38MAPK inhibition prevented both Aβ1-42-mediated down-regulation of occludin and the increase in paracellular permeability in hCMEC/D3 cells. The p38MAPK pathways might represent attractive therapeutic targets for preventing blood-brain barrier dysfunction in AD.

The accumulation of the amyloid plaques plays not only a key role in the pathology of AD, but is closely relevant to the clinical symptoms of AD, such as progressive cognitive decline, loss of memory and decreased mental capacity. Consequently, reducing the Aβ in the brain has been a primary focus in the treatment of AD. Amyloid beta peptides may be neurotoxic during the progression of AD by eliciting oxidative stress. Antioxidants prevent either neuronal apoptosis or activation of p38MAPK elicited by Aβ. However, at present the mechanism by which overproduction of Aβ peptide leads to an inflammatory response in the CNS remains poorly understood.

From previous studies, we know that oxidative stress play an important role in the increase of activation of p38MAPK. Therefore, we checked whether allicin affects the upregulated p38MAPK induced by Aβ(1-42) treatment by immunohistochemistry and Western blotting. Our study showed that brain oxidative stress, measured as SOD activity, decreased and MDA levels increased in Aβ(1-42) infused mice compared with the sham-operated mice. Furthermore, the concomitant administration of allicin increased SOD activity and decreased MDA levels, which is consistent with its known antioxidant activity in brain. We also found that allicin decreased the amyloid deposition. This decrease was coincidental with a significant reduction of the activation of p38MAPK. Our Morris Water-Maze results show that allicin improves learning and memory impairment in an Aβ(1-42) infused animal model. However, the mechanism by which amyloid deposition is decreased and p38MAPK is activated by concomitant treatment with allicin remains to be further studied.

In summary, despite the limitation of mechanisms, learning and memory deficits were reversed by concomitant treatment with allicin by reducing amyloid deposition, and activation of p38MAPK. This effect may be derived from the inhibitory effect of allicin on oxidative stress. Our results provide strong evidence that allicin may have potential protective effects on learning-memory impairments in clinical patients. The observed enhancement of learning and memory ability and reduction of Aβ deposition, scavenging free radicals after pretreatment with allicin encourages the further study of the protective effect potential on the incidence and progression of AD.

**References**

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