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

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ABSTRACT

الأهداف: التحقق من طريقة عمل مادة الأليسين (allicin) ومدى فعاليتها في حماية مهارات التعلم والذاكرة لدى مجموعة من الفئران المُصابة بمرض الزهايمر.

الطريقة: حدثت هذه الدراسة في معهد جيشو الصحي في جامعة جيشو، جيشو، الصين وذلك خلال الفترة من يناير 2009م إلى سبتمبر 2009م. لقد تم حقن مادة الألسين للتحكم بمرض الزهايمر الذي تم تحفيزه بواسطة الحقن ببروتين بيتا النشواني (amyloid beta 1–42)، وبعد ذلك تم التحقق من فعالية الأليسين في الحماية من تدهور مهارات التعلم والذاكرة. شملت هذه الدراسة 60 فئراً تم تقسيمهم عشوائياً إلى 3 مجموعات وهي كالتالي: مجموعة التحكم التي تم إعطاؤها محلول الفوسفات المنظم فقط، والمجموعة التي أُعطيت هذا المحلول مع حقنها ببروتين بيتا النشواني (1–42)، والمجموعة المُعالجة التي تم حقنها ببروتين بيتا النشواني (1–42) مع حقنها بمادة الأليسين. لقد تم حقن بروتين بيتا النشواني (1–42) (1 ميكرولتر=4 ميكروجرام) داخل الحصين الثنائي، في حين تم إعطاء محلول الفوسفات المنظم لمجموعة التحكم، وبعد ذلك تم حقن مادة الأليسين ومحلول الفوسفات المنظم داخل الصفاق لمدة 14 يوماً. لقد تم تدريب الحيوانات على هذه التجربة واُستخدمت متاهة موريس المائية (Morris Water–Maze) لاختبار مهارات التعلم والذاكرة لدى المجموعات الثلاثة. لقد تم تسجيل التغيرات التي طرأت على كيناز البروتين المُفعل بالميتوجين (p38 Mitogen Activated Protein kinase) خلال التجربة من أجل التعرف على فعالية الأليسين في حماية مهارات التعلم والذاكرة من التدهور.

النتائج: لقد كانت ردة فعل الفئران الحسية للمؤثر (latency) في المجموعة التي تم حقنها ببروتين بيتا النشواني (1–42) ومادة الأليسين أسرع بكثير من ردة فعل المجموعة التي تم حقنها بمحلول الفوسفات المنظم مع بروتين بيتا النشواني (1–42) ابتداءً من اليوم الثاني من جلسات التدريب (p=0.031)، وقد صاحب ذلك انخفاض في محتوى ألدهايد مالونديايهايد (malondialdehyde) (p=0.035) وزيادة في نشاط إنزيم فوق الأكسيد ديسموتاز (superoxide dismutase) بالدماغ (p=0.041). وقامت مادة الأليسين أيضاً بتقليل معدلات بروتين بيتا النشواني وكيناز البروتين المُفعل بالميتوجين في قشرة مخ الفئران المُصابة بمرض الزهايمر (p=0.031).

خاتمة: أشارت الدراسة إلى دور مادة الأليسين الفعال في حماية مهارات التعلم والذاكرة من التدهور، وقد يرجع ذلك إلى قدرتها على زيادة نشاط إنزيم فوق الأكسيد ديسموتاز، وتقليل مستويات ألدهايد مالونديايهايد، بالإضافة إلى تقليل مستويات بروتين بيتا النشواني وكيناز البروتين المُفعل بالميتوجين في الدماغ.

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, China, between January and September 2009. Allicin was given as preventive administration after AD was induced by amyloid beta (Aß[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 Aß(1-42)+PBS group, and the Aß(1-42)+allicin group. The Aß(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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Alzheimer's 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.[1-3] 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,[4] 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,[5] acute brain infarction,[6] and amyotrophic lateral sclerosis.[7] At present, only a few studies are focused on the therapeutical potential of allicin in vascular dementia,[8] 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,[9] 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 Aß(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 Aß(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 Aß(1-42) from US Peptide (Sigma, St. Louis, MO, USA). Anti-β-Actin, anti-p38, anti-Aß(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 Aß(1-42)+PBS group, and the Aß(1-42)+allicin group. The Aß(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.[6] 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°). 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), Aß(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 Aß(1-42) or sham-operated groups. The Aß(1-42)-infused allicin-treated group showed shorter latency times than the PBS-treated Aß(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 Aß(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 ±

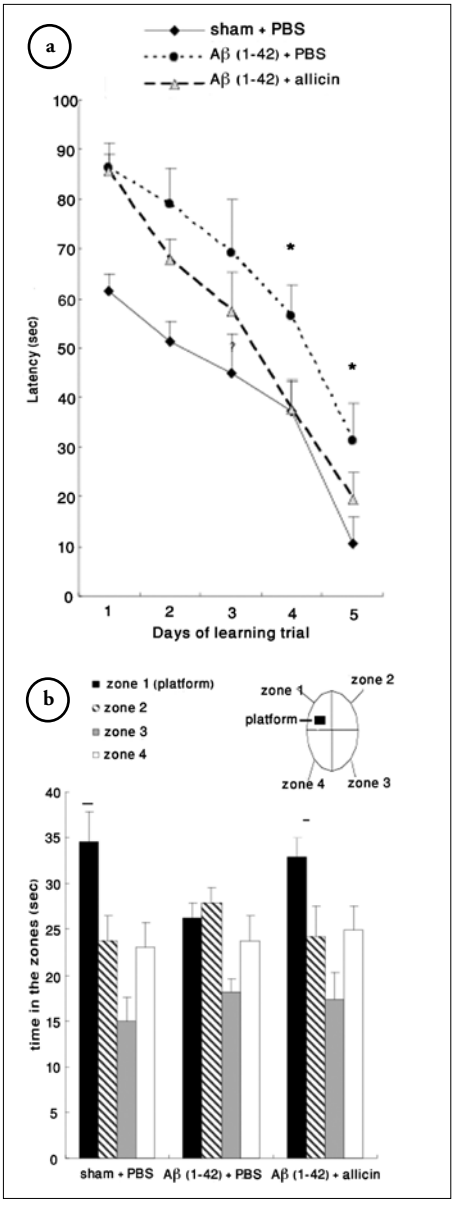

Figure 1 - Effect of allicin on the impairment of spatial memory induced by amyloid beta (Aß[1-42]) injection showing: **a**) escape latencies per group of 4 trials tested in a Morris Water-Maze over 5 days. Latency times for the animals in the allicin-treated group were compared to the Aß(1-42))injection group (*p=0.031 versus vehicle). **b**) The probe test was performed after the final training session. The times that mice of the allicin injected group stayed in zone one were compared to the Aß(1-42) injection group (*p=0.023 versus vehicle).

2.09) in zone one than at the other zones (zones 2-4) (p=0.023, **Figure 1b**). 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 Aß(1-42) infused groups, and 18.3% in the allicin-treated Aß(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 Aß(1-42) infused groups, and 30.4% in the allicin-treated Aß(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 Aß(1-42) injection group. In the allicin group, the MDA content also decreased from 71.08 ± 11.69 nmol/mg protein in the Aß(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 Aß(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 Aß were investigated by immunohistochemistry

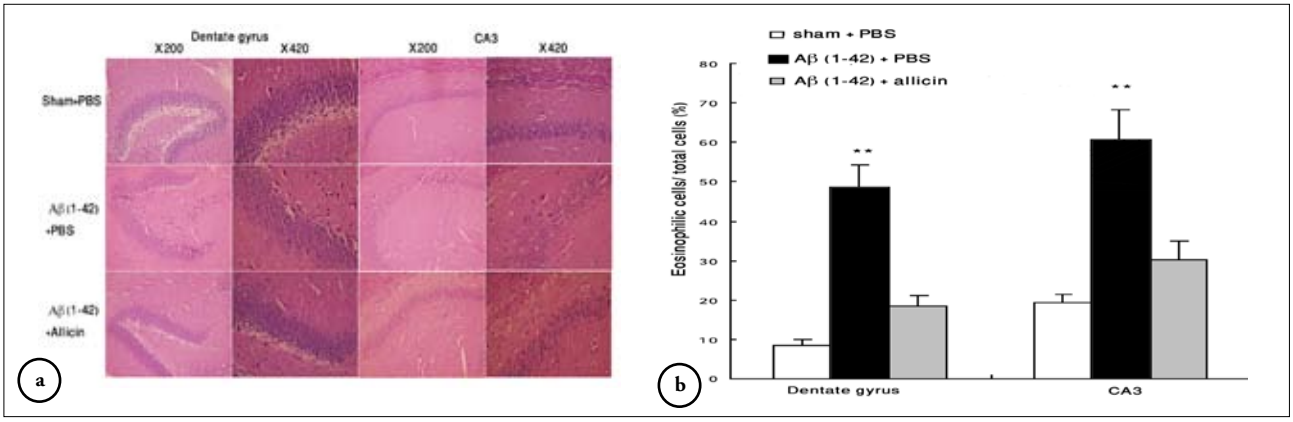

Figure 2 - Allicin reduced neuronal death in the amyloid beta (Aß[1-42]) infused Alzheimer disease (AD) mice model showing: **a**) Hematoxylin and eosin-stained sections of the hippocampal areas (dentate gyrus, CA3) of phosphate buffer saline (PBS) or allicin administered group after Aß(1-42) infusion were observed. Note that the depth of staining of neurons was reduced by allicin pretreatment in Aß(1-42) infused mice. **b**) The percentage of eosinophilic cells versus total cells was calculated in the dentate gyrus and CA3 of the PBS or allicin administered or Aß(1-42) infused rats (**p=0.005 versus vehicle).

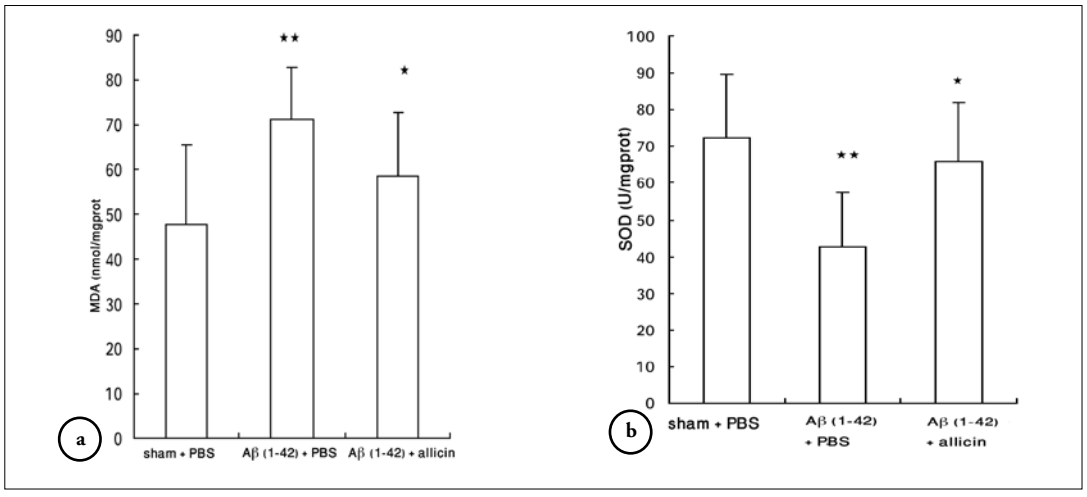

Figure 3 - Effects of allicin on **a**) malondialdehyde (MDA), and **b**) superoxide dismutase (SOD) in brains of Alzheimer disease' mice induced by amyloid beta (Aß)[1-42]) injection. **p=0.036 versus normal group, *p=0.034 versus the Aß(1-42) injection group. PBS - phosphate buffer solution

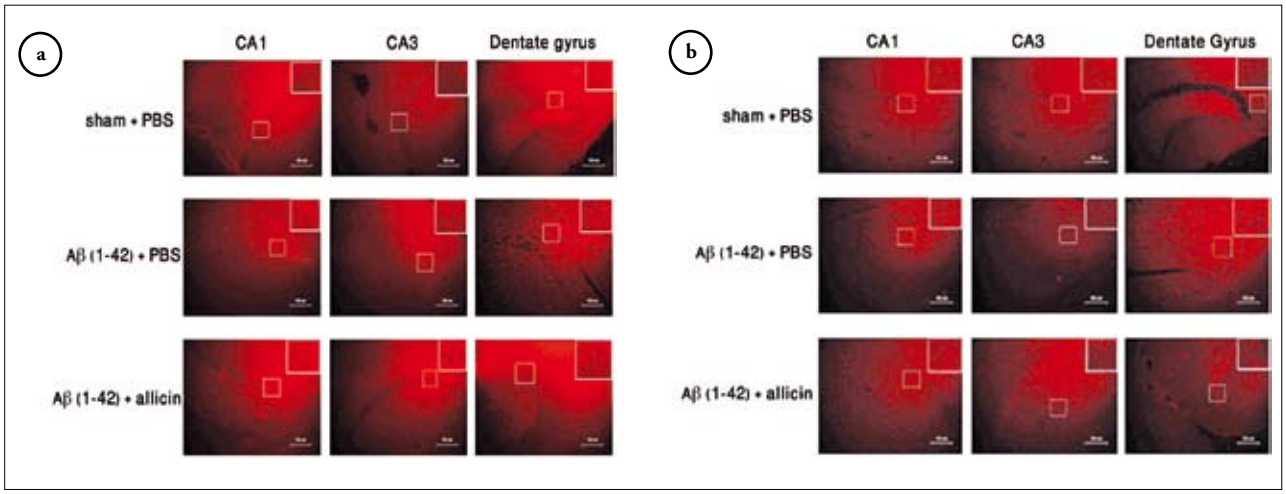

Figure 4 - Immunohistochemical evaluation of the effects of allicin on the expression of p38 mitogen-activated protein kinase (p38MAPK) and amyloid beta (Aß[1-42]) (n=5, original magnification x200) showing: **a**) the expression of Aß(1-42) in the hippocampus and dentate gyrus. **b**) The expression of p38MAPK in hippocampus and dentate gyrus. The fluorescent immunohistochemistry was performed in CA1 and CA3 and dentate gyrus with p38MAPK or Aß(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.

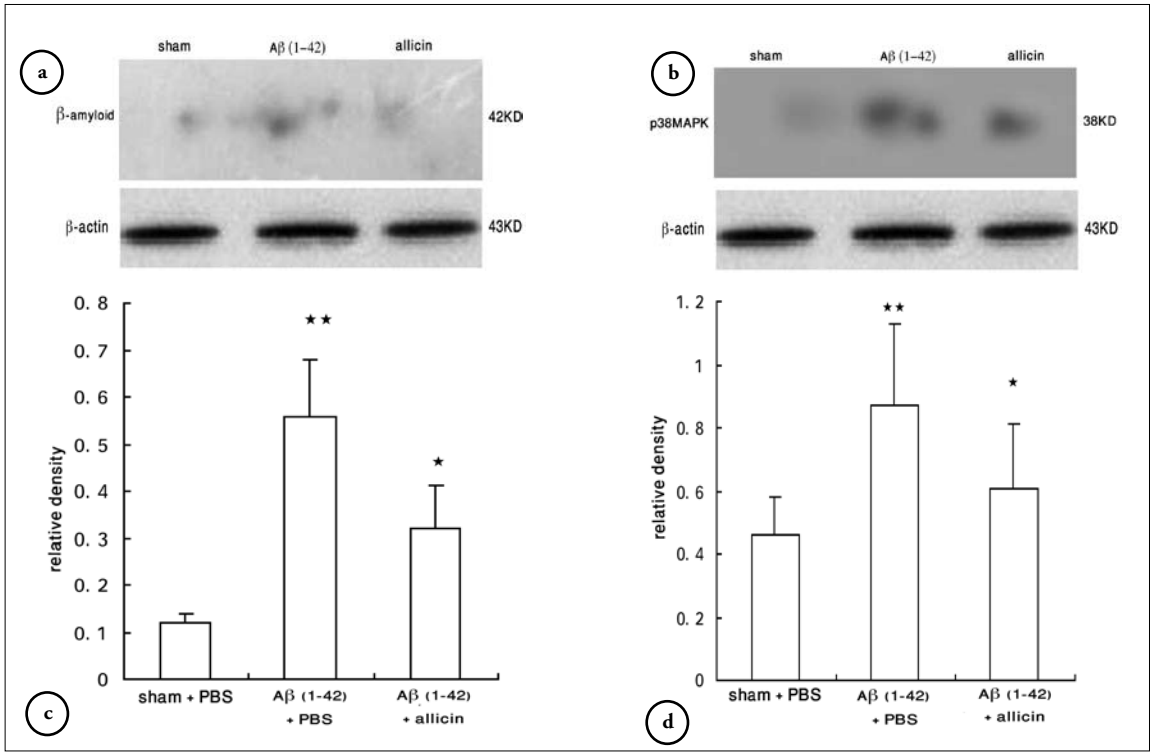

Figure 5 - Western blotting evaluation of the effects of allicin on the expression of p38 mitogen-activated protein kinase (p38MAPK) and amyloid beta (Aß[1-42]) showing: **a**) the expression of Aß(1-42) in the brain of the 3 groups. **b**) The expression of p38MAPK in the brain of the 3 groups. **c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

after the allicin treatment (180 mg/kg) for 14 days. To examine the in situ distribution of p38MAPK and Aß, sections from the hippocampus of control and Aß(1-42) infused mice were stained with p38MAPK or Aß-specific antibody. The immunoreactivities of p38MAPK and Aß in the CA1 and CA3 of the hippocampus and that dentate gyrus of Aß(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 Aß(1-42) having been infused into mice lateral ventricles continuously for a week significantly reduced

the p38MAPK immunoreactivities (**Figure** 4) (data not shown). The Aß is reported to increase p38MAPK activation in neuronal cells.[10,11] We investigated the effects of allicin on p38MAPK activation caused by Aß(1-42). Results from Western blots showed that allicin attenuated activation of p38MAPK induced by Aß(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 Aß(1-42) deposit in vivo.[10] The p38MAPK was significantly activated in microglia, astrocytes and neurons, around and distant from the plaques in neurodegenerative diseases 8 (TgCRND8) mice expressing a double mutant form of human amyloid precursor protein, and representing a good model of Alzheimer's disease.[12] The activation of p38MAPK by Aß 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 Aß peptide.[6] Meanwhile, p38MAPK inhibition prevents Aß(1-42)-mediated down-regulation of occludin.[4] Amyloidß25-35 can activate p38MAPK signal transduction pathways and lead to the increased expression of p38MAPK in the olfactory bulb of rats with AD.[13] The p38MAPK inhibition prevented both Aß1-42-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,[12] but is closely relevant to the clinical symptoms of AD, such as progressive cognitive decline, loss of memory and decreased mental capacity.[13-15] Consequently, reducing the Aß 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 Aß.[11] However, at present the mechanism by which overproduction of Aß peptide leads to an inflammatory response in the CNS remains poorly understood.

From previous studies,[11] 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 Aß(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 Aß(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 Aß(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 Aß 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

1. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, et al. Oxidative damage is the earliest event in Alzheimer disease. ***J Neuropathol Exp Neuro*** 2001; 60: 759-767.
2. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. ***Nucleic Acids Res*** 2007; 35: 7497-7504.
3. Eftekharzadeh B, Maghsoudi N, Khodagholi F. Stabilization of transcription factor Nrf2 by tBHQ prevents oxidative stress-induced amyloid beta formation in NT2N neurons. ***Biochimie*** 2010; 92: 245-253.
4. Tai LM, Holloway KA, Male DK, Loughlin AJ, Romero IA. Amyloid-beta-induced occludin down-regulation and increased permeability in human brain endothelial cells is mediated by MAPK activation. ***J Cell Mol Med*** 2010; 14: 1101-1112.
5. Zheng YH, Chen CH. Protective effects of allicin on acute cerebral ischemia-reperfusion injury in rats. ***Chinese Pharmacological Bulletin*** 2004; 20: 821-824.
6. Zhang JL, Sun RJ, Shi ZX. [Effect of garlicin on adhesion molecules expression and deformability of peripheral neutrophils in patients with acute cerebral infarction] ***Zhongguo Zhong Xi Yi Jie He Za Zhi*** 2002; 22: 423-425. Chinese.

7. Li CY, Liu XY, Bu H, Li Z, Li B, Sun MM, et al. Prevention of glutamate excitotoxicity in motor neurons by 5,6-dihydrocyclopenta-1,2-dithiole-3-thione: implication to the development of neuroprotective drugs. ***Cell Mol Life Sci*** 2007; 64: 1861-1869.
8. Wang JZ, Gao D, Gao C, Yao GE, Zhou HJ, Zhang LL, et al. Changes of interleukin-1B and TNF-A in the hippocampus and the interventional effect of garlicin on vascular dementia in rats. ***Acta Academiae Medicinae Militaris Tertiae*** 2004; 26: 1515-1517.
9. Choi Y, Kim HS, Shin KY, Kim EM, Kim M, Kim HS, et al. Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer's disease models. ***Neuropsychopharmacology*** 2007; 32: 2393-2404.
10. Giovannini MG, Scali C, Prosperi C, Bellucci A, Vannucchi MG, Rosi S, et al. Beta-amyloid-induced inflammation and cholinergic hypofunction in the rat brain in vivo: involvement of the p38MAPK pathway. ***Neurobiol Dis*** 2002; 11: 257-274.
11. Tamagno E, Robino G, Obbili A, Bardini P, Aragno M, Parola M, et al. H2O2 and 4-hydroxynonenal mediate amyloid beta-induced neuronal apoptosis by activating JNKs and p38MAPK. ***Exp Neurol*** 2003; 180: 144-155.
12. Bard F, Barbour R, Cannon C, Carretto R, Fox M, Games D, et al. Epitope and isotype specificities of antibodies to beta -amyloid peptide for protection against Alzheimer's disease-like neuropathology. ***Proc Natl Acad Sci U S A*** 2003; 100: 2023-2028.
13. Zhao L, He M, Jin WB, Zhao HS, Yao WF, Wei MJ. Vitamin E administration improves learning and memory deficits in modeling Alzheimer's disease. ***Chin J Clin Pharmacol Ther*** 2009; 14: 25-31.
14. Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. ***Neurology*** 2005; 64: 129-131.
15. Talebi M, Farhodi M, Nikanfar M, Majidi J, Fakhari A. Study on serum homocysteine level in Alzheimer's disease and its relationship with the stages of this disease. ***Neurosciences*** 2008; 13: 359-362.

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