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Allicin ameliorates cognitive deficits ageing-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways

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ABSTRACT

Numerous studies have demonstrated that learning and memory abilities in the course of normal aging cognitive abilities tend to decline and Nrf2 expression appears to decline with aging. So, Nrf2 pathway has been identified as a promising therapeutic target for neurodegenerative diseases. The organosulfur compounds, allicin can activate Nrf2, because it has an electrophilic center, which can serve as an attack site for nucleophiles, such as specific protein sulfhydryl groups present on Keap1. However, the influence of allicin on aging-induced cognitive deficits has not been examined. In this study, we assess the effects of allicin on endogenous antioxidant defenses in hippocampus of cognitively impaired aged mouse. Our results indicate that treatment of allicin significantly ameliorated ageing-induced cognitive dysfunction through enhancing of Nrf2 antioxidant signaling pathways. Therefore, allicin could be recommended as a possible candidate for the prevention and therapy of cognitive deficits in aging and Alzheimer's disease.

Aging is a group of inevitable processes involving a variety of progressive physiological and pathological changes, enhanced vulnerability to a number of diseases, including alterations in memory and cognitive function, as noted particularly in Alzheimer's disease [20]. Numerous studies have demonstrated that learning and memory abilities in the course of normal aging cognitive abilities tend to decline [15,25]. Oxidative stress (OS) is a proposed mechanism for age-related degenerative processes as a whole [1]. Humans and animals show increased cognitive declines with aging that are thought to be due to a decline in the endogenous antioxidant defense mechanisms and to the vulnerability of the brain to the deleterious effects of oxidative damage [4,3,17,12]. Therefore, it would seem that age-related deleterious effects on behavior and brain function could be retarded or even reversed by increasing antioxidant levels.

The transcription factor, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), regulates the expression of these key antioxidants, including γ-glutamylcysteine synthetase (γ-GCS), the rate limiting enzyme in the synthesis of glutathione (GSH) and NAD(P)H:quinone oxidoreductase 1 (NQO1) by recognizing the human Antioxidant Response Element (ARE) binding site within their promoter regions and defends the cell against free radical-induced damage [8]. As a result, this pathway has been identified as a promising therapeutic target for neurodegenerative diseases [23,2].

The organosulfur compounds, allicin and L-sulforaphane, can activate Nrf2, because each of these compounds has an electrophilic center which can serve as an attack site for nucleophiles, such as specific protein sulfhydryl groups present on Keap1 [5,11,9]. Allicin is the most important lipid-soluble chain breaking natural antioxidant in mammalian cells and is able to cross the blood–brain barrier and accumulate at therapeutic levels in the brain, where it reduces lipid peroxidation. Aside from the beneficial properties previously mentioned, we and other researchers have found that allicin is able to ameliorate the learning and memory impairment in the cognitively impaired individuals, such as Aβ-induced memory impairment mice, 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 [18,10]. The data suggest that the antioxidant actions of allicin may have an important role in the cognitive deficits. Therefore, it is certainly to be expected that the role of allicin in learning and memory for the cognitively impaired aging individuals may be mediated through inducers of endogenous antioxidant defenses in memory-related brain regions. In this study, we assess the effects of allicin on endogenous antioxidant defenses in hippocampus of cognitively impaired aged mouse.

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 cared for in accordance with the Guiding Principles in the Care and Use of Animals. One hundred 20-month-old male C57BL/6 mice were obtained from Sino-British Sippr/BK (Shanghai, China). Forty young male controls were obtained at 3 months of age from the same supplier. The mice

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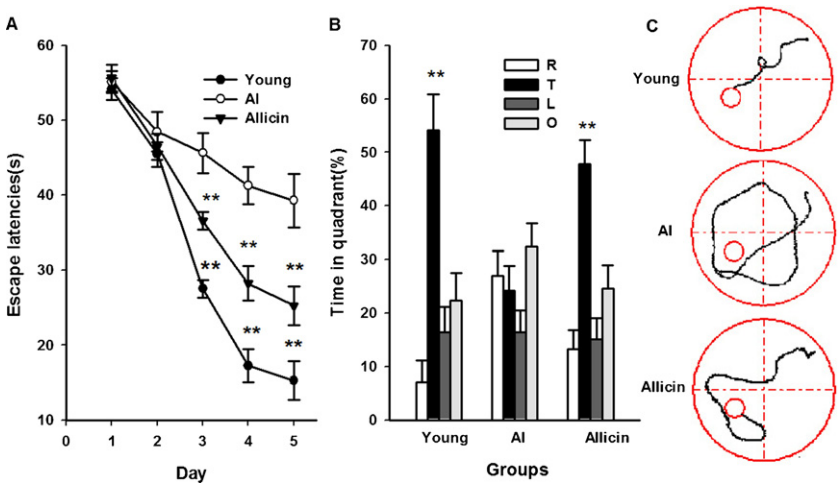

Fig. 1. Effects of allicin on aging-induced cognitive dysfunction. Young: the young group; AI: aged impaired group; allicin: allicin group. (A) AI group mice exhibited significantly higher escape latency on Days 3–5 during training trials compared to young group mice ($p < 0.01$). Allicin treatment significantly decreased escape latency compared to AI group mice on Days 3–5 ($p < 0.01$). (B) Swimming time spent in each quadrant in the probe trial on Day 6 (T, target; L, left; O, opposite; R, right). Allicin treatment significantly prevented the memory impairment, as indicated by the increase in the time spent in target quadrant ($p < 0.01$). (C) Representative path tracings of the probe test. Each value represents mean ± S.E.M. for 12–15 observations, $^{**}p < 0.01$ vs. AI group.

were housed 4 per cage with free access to food and water, and were kept in a constant environment (22 ± 2 °C, 50 ± 5% humidity, 12-h light/dark cycle). Aged mice were screened for memory impairment by Morris water maze test. The cognitive status of an aged mouse was determined on the basis of its escape latencies on Days 3–5 of testing in Morris water maze relative to the mean latency of young mice. An aged impaired (AI) mouse were defined as one whose mean latency (across Days 3–5 of testing) differed by >3.0 SDs from that of young controls [26,19].

The young control group mice ($n = 16$) were randomly selected from the young mice. The AI mice were randomly subdivided into two groups consisting of 16 animals each: AI group and allicin groups. At the time of the experiment, the young mice were 4 months of age, and the aged mice were 21 months of age. The young control group and AI group were fed on a standard rat chow (60% carbohydrate, 20% protein, 10% vitamin and mineral mix, 5% fat and 5% cellulose), while allicin group were given a diet containing 180 mg/kg/day of allicin (based on our previous work) for 8 consecutive weeks [10].

After 7 weeks of treatment, the learning and memory abilities of mice in different groups were assessed by Morris water maze test [24,14] on the 8th week. The water-maze apparatus (Chinese Academy of Medical Sciences, Institute of Materia Medica, Beijing, China) consisted of a large circular pool (122 cm in diameter, 50 cm in height, filled to a depth of 30 cm with water at 22 ± 1 °C), which was divided into four equally spaced quadrants (N, E, NW, and SE). The water was made opaque with white colored dye. A circular platform (10 cm in diameter) was placed at the SW quadrant 1 cm below the surface of the water. Each mouse was subjected to four consecutive trials on each day with a gap of 5 min and allowed 120 s to locate the submerged platform. Then, it was allowed to stay on the platform for 15 s. If it failed to find the platform within 120 s, it was guided gently onto the platform and allowed to remain there for 15 s. Escape latency time was noted as the index of acquisition or learning. The mice were subjected to acquisition trials for five consecutive days. The platform was removed and each mouse was allowed to explore the pool for 30 s on the sixth day. The time spent in all four quadrants was noted as the index of retrieval. All the trials were completed between 09.00 and 17.00 h.

After completion of water maze test, all animals were killed by decapitation under anesthesia. The brain was immediately removed and washed with ice-cold normal saline and the hippocampus was dissected. Four animal samples in each group were randomly selected and frozen in liquid nitrogen and stored at −80 °C for Western blot analysis. Hippocampus of the remaining animal samples were weighed and a 5% homogenate was prepared in a 50 mmol/l phosphate buffer (pH 7.0) containing 0.1 mmol/l EDTA. The homogenate was divided into two parts. One part was centrifuged at 3500 rpm for 10 min at 4 °C for the total antioxidant capability (T-AOC), GPx, GSH, thiobarbituric acid reactive substances (TBARS) test. The other part of homogenate was centrifuged at 12,000 × g for 10 min and the supernatant was used to determine hippocampal ROS and protein carbonyl levels.

For the determination of GPx activity, the homogenate supernatant mixed with GSH and hydrogen peroxide was incubated at 37 °C for 3 min, followed by the addition of 10% trichloro acetic acid (TCA). After centrifugation, the supernatant was collected and mixed with disodium hydrogen phosphate and 5,5-dithiobis(2-nitro-benzoic acid) (DTNB). The absorbance at 412 nm was recorded for the calculation of GPx activity. The unit of GPx activity was expressed as micromoles GSH oxidation per min per mg protein [26].

The T-AOC, GSH and TBARS were assayed with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The GSH concentration was calculated using a GSH standard curve. The plasma TBARS concentration was expressed as TBARS in nanomoles per mg protein, using a standard curve as a reference with a known quantity of malondialdehyde.

ROS was measured as described based on the oxidation of 2′,7′-dichlorodihydrofluorescein diacetate to 2′,7′-dichlorofluorescein (DCF). Briefly, the reaction mixture containing Locke's buffer, homogenate and 5 mM 2′,7′-dichlorodihydrofluorescein diacetate was incubated for 45 min at room temperature, then, which was measured using a spectrofluorimeter with excitation at 484 nm and emission at 530 nm. Background fluorescence was corrected by the inclusion of parallel blanks. ROS formation was quantified from a DCF standard curve and the data are expressed as pmol DCF formed/min/mg protein [13].

Protein carbonyls were quantified by monitoring the absorbance 370 nm in a quartz 96 well plate using a spectrophotometer, and calculated using extinction coefficient of 21.0 mM/cm for aliphatic hydrazones to calculate the amount of protein carbonyls in terms of nmol/mg protein.