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Research article

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In vitro neuroprotective effects of allicin on Alzheimer's disease model of neuroblastoma cell line

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Ethics Committee Approval
Current study does not include any experiments with human participants or animals performed by any of the authors. Since SH-SY5Y cells are considered a cell-line, the ethical concerns associated with primary human neuronal culture are not involved with these cells.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Alzheimer's disease is a progressive disorder that causes atrophy and neuronal death in the brain. Currently, there is not any effective therapy for Alzheimer's disease. The current research was designed to investigate the beneficial effects of allicin on Alzheimer's disease in SHSY-5Y cells in vitro and elucidating the neuroprotective mechanism of allicin.

Methods: Human neuroblastoma cell line (SH-SY5Y) was differentiated with retinoic acid to conduct the in vitro Alzheimer's Disease model. Amyloid β1-42 protein was applied to the cells for 24 hours (2.5 µM) to induce cytotoxicity. Allicin was applied to the cell cultures in a wide spectrum dose (10 µM, 50 µM, 100 µM) to investigate neuroprotective effect against amyloid β for 24 hours. MTT and LDH analyses were performed to assess the cell viability. MDA and ROS concentrations and SOD activity were analyzed to determine the oxidative stress. Moreover, the effects of allicin on the caspase-3 expression in amyloid β induced neurotoxicity were determined by the RT-PCR analysis.

Results: Amyloid β markedly decreased cell viability of SH-SY5Y in MTT analysis and elevated LDH levels. In contrast, in MTT analysis, the allicin markedly increased cell viability, indicating that allicin induces cell proliferation. Moreover, in LDH analysis, allicin treatment markedly decreased LDH release. Exposure to amyloid β markedly increased MDA and ROS levels, in comparison with the control. Moreover, amyloid β decreased activity of SOD in SH-SY5Y cells. Allicin markedly balanced out the amyloid β-induced MDA and ROS generation. In the same pathway, allicin increased activity of SOD in amyloid β -exposed SH-SY5Y cells. The caspase 3 expression was increased in amyloid β group in comparison to the control group and allicin markedly lowered the expression of caspase-3 levels.

Conclusion: The beneficial effects of allicin on amyloid β -induced neurotoxicity on SH-SY5Y cells were reported for the first time in terms of cell viability, oxidative stress and apoptosis.

Keywords: Allicin, Alzheimer's disease, Antioxidant, Apoptosis, SH-SY5Y cell line

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Introduction

Alzheimer's disease (AD), a progressive neuro-degenerative disease with a globally increasing prevalence, results from the aggregation of amyloid β (Aβ) protein and hyperphosphorylated Tau proteins, causing interruption of synaptic connections and neuronal-death [1, 2]. Patients with AD develop not only dementia, but also memory loss, difficulties in daily routine activities, and also psychological abnormalities such as depression. Pathophysiological characteristics of AD involve amnesia and cognitive deterioration [3].

Lesions of the cerebral cortex and hippocampus are found in neurons with abnormally hyperphosphorylated neurofibrillary tangles and extracellular Aβ plaques [4, 5]. Among these AD symptoms, the development of senile plaques and the accumulation of neurofibrillary tangles are considered as triggers in neuro-degenerative disorders. In fact, senile plaques often occur because of Aβ deposition in neuronal and glial cells. Aβ aggregation can cause oxidative damage, cell membrane/cell cycle disturbances, protein folding defects and DNA damage [6].

One of the contributing factors in most neuro-degenerative diseases is age and age might be connected with oxidative damage which enhances with age and participates in the pathology of neurodegeneration. When redox balance disrupts, oxidatively modified molecules accumulate in neuronal cells and cause dysfunction [7]. In extremely sensible cells like neurons, cell death may occur as a result of limited defense systems and failure [8]. The increase in chronic neuroinflammation also takes an important place in the pathophysiology of AD. Overexpression of proinflammatory cytokines was also associated with neuronal loss by increased Aβ aggregation and hyperphosphorylation of Tau [9]. In addition, abnormal accumulation of Aβ accelerates the inflammatory process by increasing proliferation and activation of microglial cells and increases the release of proinflammatory cytokines [9,10].

As an antioxidant, anti-inflammatory, neuroprotective and anti-cholinesterase molecule, allicin is being tested as a physiologically active compound due to its therapeutic potential in some neurological diseases [11]. It improves mitochondrial functioning, thus preserving neuronal cells by settling the cellular oxidative stress and reducing apoptosis [12]. It is also reported that allicin has an antioxidative effect and protects the brain from ischemia damage [13].

Current research is the first to examine the impacts of allicin against Aβ1- 42 induced AD model in vitro. The novelty of this study lies in investigating the effects of allicin on AD in SH-SY5Y cells in vitro and elucidating the neuroprotective mechanism of allicin. This study provides a new molecular treatment strategy for the clinical therapy of AD.

Materials and methods

Cell culture procedure

Human neuroblastoma SH-SY5Y cells were acquired from ATCC (USA) and cultured as manual provided by manufacture. The cells were placed in sterile 25 cm^2 flasks in DMEM (Dulbecco's Modified Eagle Medium) culture medium containing 10% FBS, 1% L-glutamine and 1% penicillin-streptomycin in a 5% carbon dioxide (CO_2) incubator. Cells were passaged with Trypsin/Ethylene Diamine Tetraacetic Acid (EDTA) when they covered 80% of the flask. Cells were differentiated with 10 µM alltrans retinoic acid for 6 days before the allicin treatment. Commercially purchased (Sigma Aldrich) lyophilized Aβ1-42 was prepared according to the recommended preincubation protocol to obtain the required peptide aggregates in neurotoxicity studies. Formation of peptide aggregates was achieved by dissolving the lyophilized peptide in 167 µl of molecular biological water. The final concentration in the vial was 1mg/ml to 1ml with 833 µl of sterile PBS. Allicin treatments (10 µM, 50 µM, 100 µM) [14] were applied to neuroblastoma cells in culture medium.

Cell viability

MTT assay

MTT test was utilized to examine the cell viability. MTT solution (Sigma-Aldrich) was supplemented to each well in accordance with the kit protocol. After incubation interval, formazan precipitate was dissolved in DMSO (150 µl) and the absorbance were read with spectrophotometer at 480 nm (BioTek Instruments, USA).

LDH assay

The cytotoxicity was evaluated via the LDH assay. Lactate dehydrogenase (LDH) levels were examined in accordance with the manufacturer's guideline (Elabscience, United States). The absorbance was read at 450 nm.

Evaluation of oxidative stress

The malondialdehyde (MDA) and reactive oxygen species (ROS) concentration and the activity of superoxide dismutase (SOD) were determined by using ELISA kits (Elabscience, United States) in conformity with kit protocol. ROS concentration was measured by using ELISA kit (LSBio, United states) according to manufacturer's guide.

RT-PCR analysis

mRNA extraction and cDNA synthesis were conducted with RNeasy easy kit (Qiagen, Hilden, Germany) as defined before [15]. cDNA was synthesized with a High-Capacity cDNA Reverse Transcription Kit in compliance with manufacturer's guidelines. Real-time RT-PCR analysis were conducted as previously defined [15]. The mRNA caspase-3 expressions were calculated by Rotor-Gene Q (QIAGEN). The mRNA expression level of each gene relative to β-actin. was determined by the $2^{-\Delta\Delta Ct}$ method. The sequences of sense and antisense primers for the human Caspase-3 was 5'- TTTTCAGTCCGGGGACAAAC-3' and 5'- GGGCAGCCGAGAATAACAAT-3'.

Statistical analysis

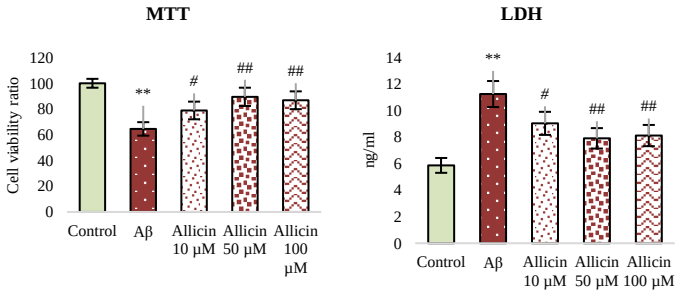
Data obtained in the study were analyzed statistically with IBM SPSS Statistics (Version 22.0, IBM Co., Chicago, IL, USA) software. All tests were conducted by one-way analysis of variance (ANOVA) with post hoc Tukey's test. The ANOVA is utilized to define whether there are any statistically significant differences between the means of independent groups. The Tukey Test is a post-hoc test designed to conduct a pairwise comparison of the means to see where a significant difference stands. Data were expressed as mean (SD). *P*-value <0.05 were assumed meaningful. The results are stated according to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

Results

MTT and LDH test results

MTT and LDH results are presented in Figure 1. Aβ markedly decreased the cell viability of SH-SY5Y by 35.46% in MTT analysis and elevated LDH levels by 91.65%. In contrast, in MTT analysis, the allicin treatment markedly increased cell viability indicating that allicin induces cell proliferation by 22.20%, 38.59% and 34.50% in cells treated with 10 µM, 50 µM and 100 µM of allicin, respectively, relative to the Aβ group. Moreover, in LDH analysis, allicin treatment markedly decreased LDH release by 19.64%, 29.68% and 27.82% in cells treated with 10 µM, 50 µM and 100 µM of allicin, respectively, relative to the Aβ group (P<0.05).

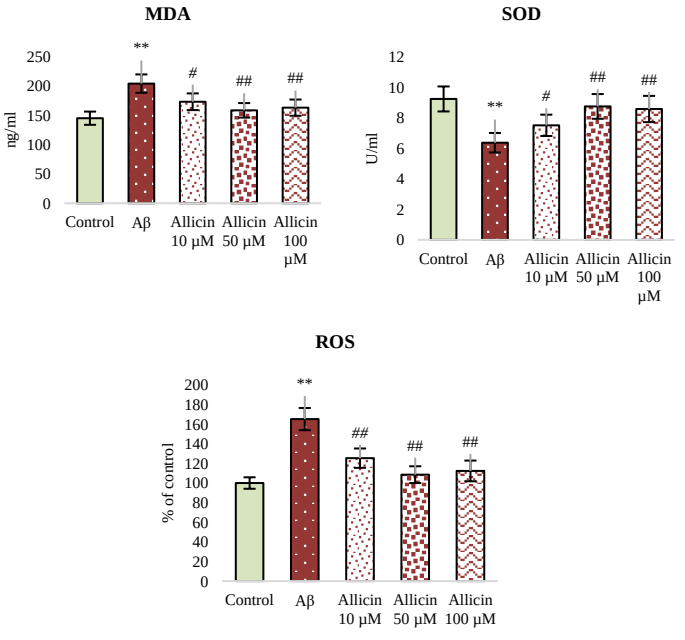
Figure 1: Effects of Allicin on cell viability test results. Data are expressed as the means (SD). ** P<0.001 vs control group, # P<0.05 vs Aβ group, ## P<0.001 vs Aβ group.



Oxidative stress results

Since the generation of free oxygen radicals and lipid peroxidation products are implicated in pathologic processes of neurotoxicity, we examined the oxidative and anti-oxidant biomarkers. Exposure to Aβ markedly increased MDA and ROS level by 40.7% and 65.21%, respectively, in comparison with the control (P<0.001) (Figure 2). Moreover, Aβ decreased the activity of SOD in SH-SY5Y cells by 31.06%. Allicin at concentrations of 50 µM markedly balanced out the Aβ-induced MDA and ROS production by 22.35% and 34.3% (P<0.001), respectively (Figure 2). Similarly, allicin at the dose of 50 µM increased the activity of SOD in Aβ-exposed SH-SY5Y cells by 37.36% (P<0.001).

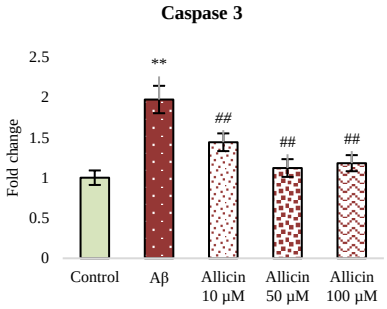
Figure 2: Effects of Allicin on oxidative stress markers. Data are expressed as the means (SD). ** P<0.001 vs control group, # P<0.05 vs Aβ group, ## P<0.001 vs Aβ group.



mRNA expression of Caspase 3

The effects of allicin on the caspase 3 expression in Aβ-induced neurotoxicity were determined by the RT-PCR analysis. Caspase 3 expression were upregulated in Aβ group by 1.97-fold in comparison to control group (Figure 3). Allicin at dose of 50 µM markedly lowered the expression of caspase 3 levels by 43.14%.

Figure 3: Effects of Allicin on apoptosis. Data are expressed as the means (SD). ** P<0.001 vs control group, ## P<0.001 vs Aβ group.



Discussion

In this study, we established neurotoxicity with Aβ on human SH-SY5Y cells to assess the protective effect of allicin in AD. Studies on AD patients have reported that Aβ plaque deposition causes cytotoxicity by increasing ROS production and oxidative stress [16]. Prevention or elimination of Aβ-mediated cytotoxicity associated with oxidative stress may be a novel and important approach for prophylactic/therapeutic purposes in AD. In recent years, natural antioxidants obtained from plants have come to the forefront to prevent oxidative damage, especially in the prevention of neuropsychiatric disorders associated with oxidative stress.

Allicin, one of the most important components of garlic, has many medicinal properties such as antioxidant and anti-apoptotic functions [13, 17]. Allicin's capability to easily cross the blood-brain barrier and its known neuroprotective properties make it worthy of investigation in neurodegenerative diseases [18-20]. Studies have shown that allicin increases SOD activity and decreases MDA levels by inhibiting signaling pathways related to oxidative damage. With these antioxidant mechanisms, allicin has shown beneficial effects in memory and learning disorders and ischemic brain damage [12, 13]. In line with previous studies, allicin significantly decreased MDA and ROS levels, and increased the SOD activity in our study. These antioxidant effects can be seen as an important reason for the positive effects of allicin on cell viability.

One of the important pathological processes associated with AD is apoptosis [21]. Activation of caspases is an important step in cellular apoptosis [22]. The caspase superfamily of cysteine proteases, including caspase 3, participates in the beginning and completion of the apoptotic process [23]. The increase in ROS production activates stress pathways associated with the mitochondria and endoplasmic reticulum. Mitochondria damage ultimately triggers the caspase 3, eventually induces mitochondria-dependent apoptosis [24]. Current research showed that allicin significantly reduced apoptosis by reducing caspase 3 expression, in accordance with previous reports.

Limitations

Although our study has strong evidences for the neuroprotective effects of allicin, it has some limitations. First of all, more studies should be done to determine whether these promising results obtained in cell culture can also be effective in sick individuals. In addition, allicin may exhibit different pharmacological properties such as antibiotic function apart from neuroprotection, and the existence of secondary effects of allicin on humans is unclear. These limitations will be eliminated with future studies on animal and human subjects to investigate the effects of allicin on AD.

Conclusion

In this study, the beneficial effects of allicin on Aβ-induced neurotoxicity on SH-SY5Y cells were reported for the first time in terms of cell viability, oxidative stress and apoptosis.

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