A Diarylheptanoid Exhibits Protective effects against Amyloid-β Induced Cytotoxicity

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Abstract

Alzheimer’s disease (AD) is a severe neurodegenerative disorder characterized by β-amyloid (Aβ) plaques, neurofibrillary tangles, neuronal death, progressive cognitive impairment and memory loss. β-amyloid peptides (Aβ) are major compositions of so-called senile plaques, which are hallmarks of AD and contribute to AD progression. Therefore, it is important to discover therapeutics against Aβ-induced toxicity. Here, we report that 7-(4-Hydroxyphenyl)-1-phenyl-4E-hepten-3-one (AO-1), a diarylheptanoid extracted from the rhizomes of Alpinia officinarum, showed effects against Aβ-induced cytotoxicity. The results demonstrated that AO-1 possesses neuroprotective activities which are capable of maintaining normal cell viability of PC12 cells and primary cortical neurons after Aβ insult.

Keywords

Alzheimer’s disease, Aβ, diarylheptanoid, neuroprotection

1. Introduction

Alzheimer’s disease (AD), one of the most common cause of dementia, is characterized by neuropathological hallmarks including β-amyloid (Aβ) plaque, neurofibrillary tangles, neuronal death, synaptic dysfunction, progressive deterioration of cognitive function and the loss of memory [1, 2]. Currently, there is no effective treatment that delays the onset or slows the progression of AD [3]. Although the precise mechanisms of AD pathogenesis are only partially understood, multiple lines of evidence have shown that the accumulation of β-amyloid peptide plays a key neurotoxic role in this disease [4, 5]. Thus, agents that can antagonize the toxicity or attenuate the formation of the toxic Aβ species are potential intervening approaches for AD [6]. In this regard, the protective effects against Aβ-duce toxicity in cultured cortical neurons or other Aβ-sensitive neuronal cell lines can be used as a primary screening assay in the search for potential leads.

The Alpinia officinarum Hance is mainly cultivated in the South China, especially Guangdong province. Its rhizomes (also named Gaolangjiang) have long been used as a spice in Europe and China and as a traditional Chinese medicine for relieving stomach ache, treating colds, invigorating the circulatory system, and reducing swelling in China [7, 8]. Previous phytochemical studies on this plant had resulted in the isolation of a number of diarylheptanoids, some of which showed antiplatelet, antioxidative, and anti-inflammatory and inhibition of pancreatic lipase activities[9, 10].

7-(4-Hydroxyphenyl)-1-phenyl-4E-hepten-3-one (AO-1; Fig.1) is one of diarylheptanoids isolated from Gaolangjiang. Our previous work revealed that AO-1 promoted neurite outgrowth in both Neuro-2a cells and cultured hippocampal neurons, which indicated that AO-1 possesses bioactivity in nervous system [11]. In this paper, we focus on whether AO-1 exhibits neuroprotection effects. By using the rat pheochromocytoma (PC12) cells and rat primary cortical neurons as models, and MTT assay as the main approach to measure cell viability, we demonstrated that AO-1 has neuroprotective potentials against Aβ42 induced cytotoxicity.
2. Materials and Methods

2.1 Reagents and chemicals

PC12 cell line was purchased from American Type Culture Collection (ATCC; Manassas, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), horse serum (HS), Neurobasal medium, B27, L-glutamine and penicillin/streptomycin were purchased from Life Technologies (Carlsbad, USA). AO-1 was extracted and purified as previously described[11]. Human Aβ42 (1-42 a.a.) peptides were purchased from rPeptide (Georgia, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), D-Glucose, poly-L-lysine and dimethylsulfoxide (DMSO) were from Sigma-Aldrich (St Louis, USA).

2.2 Aβ Oligomerization

Human Aβ42 (1-42 a.a.) peptides were oligomerized at 37 °C in a 5% CO2-supplemented atmosphere for 7 days. Soluble oligomerized Aβ42 (equivalent to 1 μM peptides) was used for the neurons experiments and (equivalent to 25 μM peptides) for the PC12 cells experiments.

2.3 Cell culture

Rat pheochromocytoma (PC12) cells were plated in 96 well plates at a density of 1×10^4/ well and cultured in DMEM supplemented with 6% FBS, 6% HS and 1% penicillin/streptomycin. Cells were allowed to differentiate in assay medium, which was consisted of DMEM containing 0.5% FBS and with the addition of 50 ng / mL Nerve Growth Factor (NGF). The assay medium was changed every other day for 7 days. Then the differentiated PC12 cells were used for experiments.

Rat primary cortical neurons were prepared from embryos of pregnant Sprague Dawley (SD) rats (18-day pregnancy) and plated on poly-L-lysine (0.1 mg / mL) coated 96 well plates (1 × 10^5/ well) as previously described[12, 13]. The neurons were cultured in Neurobasal medium supplemented with 2% B27, 1 mM L-glutamine and 1% penicillin/streptomycin. At 7 days in vitro (7 DIV), cortical neurons were used for experiments.

2.4 Cell viability

MTT assay was used to evaluate cells viability. The assay was performed in both PC12 cells and rat primary cortical neurons. PC12 cells were cultured and differentiated as described above. The differentiated PC12 cells were then subjected to Aβ42 treatment with or without AO-1 for 48h. Similarly, primary cortical neurons were prepared and cultured as described above. At 7 DIV, cells were then exposed to Aβ42 treatment with or without AO-1 for 48h. Then 30 μl MTT (5 mg / mL) was added to each well for 3 h to allow the viable cells turn MTT to a purple formazan dye. Finally, 100 μl DMSO was added to dissolve the resultant formazan product, and absorbance at 570 nm was measured by a DTX880 multimode detector (Beckman Coulter, USA).

2.5 Statistical analysis

All experiments were operated independently at least three times. Data were shown as the mean±SEM. Using one-way ANOVA followed by the Tukey's test for Statistical analysis. P < 0.05 was considered to be statistical significant.
3. Results

3.1 AO-1 protects differentiated PC12 cells from Aβ42 induced cytotoxicity.

To determine the neuroprotective effects of AO-1, we first performed MTT assay in differentiated PC12 cells, a neuron-like cell line which is widely used as an in vitro substitution of neurons for drug screening. Cells were exposed to Aβ42 with or without AO-1 (0.125, 0.25 and 0.5 μM) for 48 h (Fig. 2). The data revealed that PC12 cells treated with Aβ42 showed a decreased cell viabilities (20-25%) comparing to control, which was reversed by 0.5 μM AO-1 treatment. It demonstrated that AO-1 possesses neuroprotective activity against Aβ42 induced cytotoxicity in differentiated PC12 cells.

Fig 2. Cytoprotective effects of AO-1 in PC12 cells treated with Aβ42. Differentiated PC12 cells were subjected to Aβ42 and co-treated with AO-1 (0.125 μM, 0.25 μM or 0.5 μM) or DMSO for 48 h. Cell viabilities were determined by MTT assay. Cell viability of control cells treated with DMSO was normalized to 100%. **P<0.01, Aβ42 vs. control group; ##P<0.01, AO-1 vs. Aβ42-treated group.

Three independent experiments in each group were measured. One-way ANOVA followed by Tukey’s test. Error bars depict mean ± SEM.

3.2 AO-1 protects cultured cortical neurons against Aβ42 induced neurotoxicity.

We next examined in cultured cortical neurons to further confirm the neuroprotective effect of AO-1. Similar to the results in PC12 cells, we also observed that the cell viability of cortical neurons decreased by Aβ42 insult, and significant restoration of cell viability was induced by co-treatment of AO-1 (0.5 or 2 μM; Fig. 3). It indicated that AO-1 promoted cultured cortical neuron survival when cells were exposed to Aβ42, which confirmed the protective effect of AO-1 against Aβ42 induced neurotoxicity in neurons.

Fig 3. Neuroprotective effects of AO-1 in cultured cortical neurons treated with Aβ42. Cultured cortical neurons (7 DIV) were exposed to Aβ42 and co-treated with AO-1 (0.5 or 2 μM) or DMSO for 48 h. MTT assay were used for cell viability detection. Cell viability of control cells treated with DMSO was normalized to 100%. ###P<0.001, Aβ42 vs. control group; #P<0.05, AO-1 vs. Aβ42-treated group. Three independent experiments in each group were measured. One-way ANOVA followed by Tukey’s test. Error bars depict mean ± SEM.
4. Conclusion

Nowadays Alzheimer’s disease is the most prevalent neurodegenerative disorder in the elderly. It is important to discover drugs with the potential of curing AD or relieving the symptoms of the disease. Protection against Aβ-induced neurotoxicity was considered as a beneficial response against Alzheimer's disease [5, 6]. To demonstrate that AO-1 was capable of reducing neurotoxicity induced by Aβ42, we measured cell viabilities using MTT method in both differentiated PC12 cells and cultured cortical neurons. Aβ42 oligomers were added into cells to induce cytotoxicity, which led to a decrease of cell viability. In the presence of AO-1, however, the toxic effects of Aβ42 were largely prevented and normal cell viability levels were retained. This study demonstrates that AO-1 shown an ability of protecting neurons against cytotoxicity caused by Aβ42, thus providing new evidence on the understanding of neuroprotection of AO-1.

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References