

## Neuroprotective Effects of AO-1 and AO-2 on Amyloid $\beta$ -induced Cytotoxicity in SH-SY5Y Cells

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### Abstract

Alzheimer's disease (AD) is an age-related and progressive neurodegenerative disease, which is characterized by  $\beta$ -amyloid ( $A\beta$ ) plaques within the brain.  $A\beta$  peptides in both soluble oligomerized forms and insoluble plaques are believed as main causes of Alzheimer's disease (AD). There is by far no effective treatment for AD. Therefore, it is urgent to explore potential therapeutic options against  $A\beta$ -induced toxicity. We previously found that AO-1 (7-(4-Hydroxyphenyl)-1-phenyl-4E-hepten-3-one) and AO-2 (7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4E-hepten-3-one), two diarylheptanoids extracted from the plant *Alpinia officinarum*, have potentials to promote neurite outgrowth in both Neuro-2a cells and cultured hippocampal neurons. In this study, we investigated the neuroprotective roles of AO-1 and AO-2 against  $A\beta_{1-42}$ -induced damage in SHSY-5Y cells. Our study demonstrates that both compounds are capable of reversing  $A\beta_{1-42}$ -induced decrease of cell viability, and AO-2 exhibits stronger neuroprotective effect than AO-1.

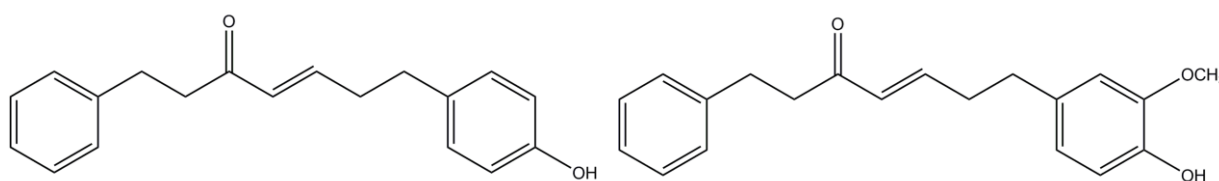
### Keywords

Alzheimer's disease,  $\beta$ -amyloid, Diarylheptanoid, Neuroprotection.

### 1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide, which is characterized by the presence of aggregates of the amyloid  $\beta$ -protein ( $A\beta$ ) [1, 2]. The accumulation of  $\beta$ -amyloid ( $A\beta$ ) is the main cause in driving AD pathogenesis [3, 4].  $A\beta_{1-42}$ , the principal component of amyloid deposits, plays a critical role in the pathogenesis of AD [5, 6, 7].  $A\beta_{1-42}$  is neurotoxic and can induce oxidative stress and other damaging effects in AD neurodegeneration [7, 8, 9]. Thus, reducing  $A\beta$ -induced oxidative stress may be an effective method to treat AD. A number of antioxidants, such as curcumin, Vitamin E and resveratrol have been reported to have protective effects against  $A\beta$ -induced cytotoxicity in cells or neurons [10, 11, 12,13].

Curcumin, a natural diarylheptanoid, has been reported to have potential to prevent Alzheimer's disease in vitro and in vivo [14, 15]. We have reported previously that two natural diarylheptanoids, AO-1 (7-(4-Hydroxyphenyl)-1-phenyl-4E-hepten-3-one) and AO-2(7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4E-hepten-3-one), promote neurite outgrowth in both Neuro-2a cells and cultured hippocampal neurons [16]. Moreover, AO-1 exhibits protective effects against  $A\beta$ -induced neurotoxicity in neurons [17, 18]. However, the protective effects of AO-1 and AO-2 on  $A\beta_{1-42}$ -induced neurotoxicity in SHSY-5Y cell have not been reported. Here, we compared the effects of AO-1 and AO-2 treatment against  $A\beta$ -induced neurotoxicity in SHSY-5Y cells by MTT assay. The results showed that AO-2 exhibits more effective neuroprotection than AO-1 on  $A\beta_{1-42}$ -induced decrease of cell viability.



AO-1: 7-(4-Hydroxyphenyl)-1-phenyl-4E-hepten-3-one;  
AO-2: 7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4E-hepten-3-one.

Fig. 1 Chemical structures of AO-1 and AO-2.

## 2. Materials and Methods

### 2.1 Reagents and chemicals

The human neuroblastoma cell line SHSY-5Y was obtained from the American Type Culture Collection (ATCC; Manassas, USA). Modified eagle medium (MEM), fetal bovine serum (FBS) and penicillin/streptomycin were purchased from Life Technologies (Carlsbad, USA). AO-1 and AO-2 were extracted and purified as previously described [16]. Human  $A\beta_{1-42}$  peptides were purchased from rPeptide (Georgia, USA). 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich (St Louis, USA).

### 2.2 Drugs preparation

$A\beta_{1-42}$  was oligomerized as described previously [17]. Briefly,  $A\beta_{1-42}$  peptides were dissolved in PBS to a concentration of 1 mM followed by the incubation under 37 °C for 7 days for aggregation. Soluble oligomerized  $A\beta_{1-42}$  (equivalent to 0.5-16  $\mu$ M peptides) was used for the SHSY-5Y cells experiments. AO-1 and AO-2 was dissolved in DMSO at a concentration of 10 mM and further diluted with medium. The final concentration of DMSO was 0.1%, which did not affect cell viability. DMSO with equal volume of the paired AO-1 or AO-2 treatment was added as the vehicle control in each experiment.

### 2.3 Cell culture

SHSY-5Y cells were cultured in Modified Eagle's medium plus 10% FBS and 1% penicillin/streptomycin. The medium was changed every 3 days. SHSY-5Y cells were seeded in 96 well plates at a density of  $2 \times 10^4$  / well.

### 2.4 Cell viability

Cell viability was performed by using the MTT assay. The cells were exposed to different concentrations of AO-1 and AO-2 for 2 h and then subjected to  $A\beta_{1-42}$  for 24 h. The cells were then incubated with 30  $\mu$ L MTT (5 mg / mL) for 4 h at 37°C. 100  $\mu$ L DMSO was added to dissolve the formazan crystals. The number of viable cells in each well was measured at 595 nm using a DTX880 multimode detector (Beckman Coulter, USA). Cell viability was showed as a percentage of the control cell culture value using the following formula: Cell viability = (absorption of sample - absorption of background) / (absorption of control - absorption of background)  $\times$  100%.

### 2.5 Statistical analysis

All data were shown as the mean  $\pm$ SEM of at least three experiments. Using one-way ANOVA followed by the Bonferroni's Multiple Comparison Test.  $P < 0.05$  was considered to be statistical significant.

### 3. Results

#### 3.1 SHSY-5Y cells were exposed to different concentrations of A $\beta$ <sub>1-42</sub> (0.5, 1, 2, 4, 8, 16 $\mu$ M) for 24 h.

We first determined the neurotoxic effects of A $\beta$ <sub>1-42</sub> on SHSY-5Y cells. Our results showed that A $\beta$ <sub>1-42</sub> induced a decrease in cell viability in a concentration-dependent manner. Cell viability was about 60% of control cultures after exposure to 4  $\mu$ M A $\beta$ <sub>1-42</sub> for 24 h, and a further decrease (35.35%  $\pm$  2.80%) was obtained after exposure to 16  $\mu$ M A $\beta$ <sub>1-42</sub> (Fig. 2). Based on these results, 4  $\mu$ M A $\beta$ <sub>1-42</sub> was used in the following experiments to assess the neuroprotective effect of AO-1 and AO-2.

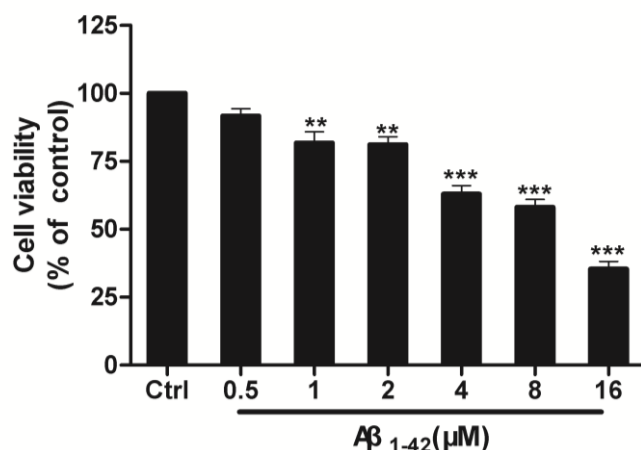


Fig. 2 Effects of different concentrations of A $\beta$ <sub>1-42</sub> on cell viability. MTT assay showed that A $\beta$ <sub>1-42</sub> at low concentration (0.5  $\mu$ M) does not affect the viability of SHSY-5Y cells, but 1-16  $\mu$ M affect the viability of SHSY-5Y cells. \*\*P<0.001, A $\beta$ <sub>1-42</sub> vs. control group; \*\*\*P<0.0001, A $\beta$ <sub>1-42</sub> vs. control group. Values were obtained from three experiments and shown as mean  $\pm$  SEM (% of control).

#### 3.2 Effects of gradient concentrations of AO-1 and AO-2 on cell viability.

To determine the safe concentrations of AO-1 and AO-2, SHSY-5Y cells were exposed to AO-1 or AO-2 with increasing concentrations from 0.5  $\mu$ M to 16  $\mu$ M for 24 h. As shown in Fig. 2, treatment with AO-1 or AO-2 (8  $\mu$ M and 16  $\mu$ M) led to a strong decrease in cell viability. The cell viability is below 90% of the control when 4  $\mu$ M of AO-1 was treated. So we used 1  $\mu$ M and 2  $\mu$ M AO-1 or AO-2 for the next experiments.

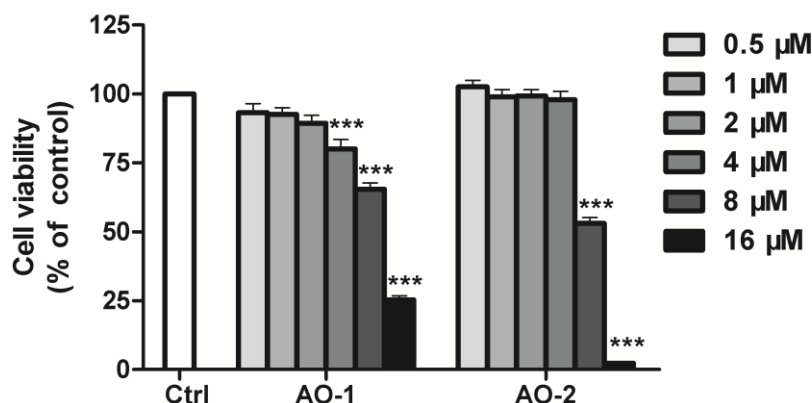


Fig 3. Test of gradient concentrations of AO-1 and AO-2 on cell viability. SHSY-5Y cells were treated with AO-1 or AO-2 (0.5, 1, 2, 4, 8, 16  $\mu$ M) for 24 h. DMSO was added as the control (Ctrl). \*\*\*P<0.001 vs. control group. Values were obtained from three independent experiments and expressed as mean  $\pm$  SEM (% of control).

### 3.3 AO-1 and AO-2 protected SHSY-5Y cells from A $\beta$ <sub>1-42</sub>- induced neurotoxicity.

SHSY-5Y cells were pretreated with AO-1 or AO-2 for 2 h before treatment with 4  $\mu$ M A $\beta$ <sub>1-42</sub> for another 24 h. The MTT assay showed that 1  $\mu$ M AO-1 does not reverse the viability of SHSY-5Y cells induced by A $\beta$ <sub>1-42</sub>, but 2  $\mu$ M AO-1 improve the viability of SHSY-5Y cells. By contrast, both 1  $\mu$ M and 2  $\mu$ M AO-2 significantly reduced A $\beta$ <sub>1-42</sub>-induced toxicity in SHSY-5Y cells, and the effects were significantly better than AO-1 at same concentrations. These results indicate that AO-2 has more potential to promote the survival of SHSY-5Y cells than AO-1.

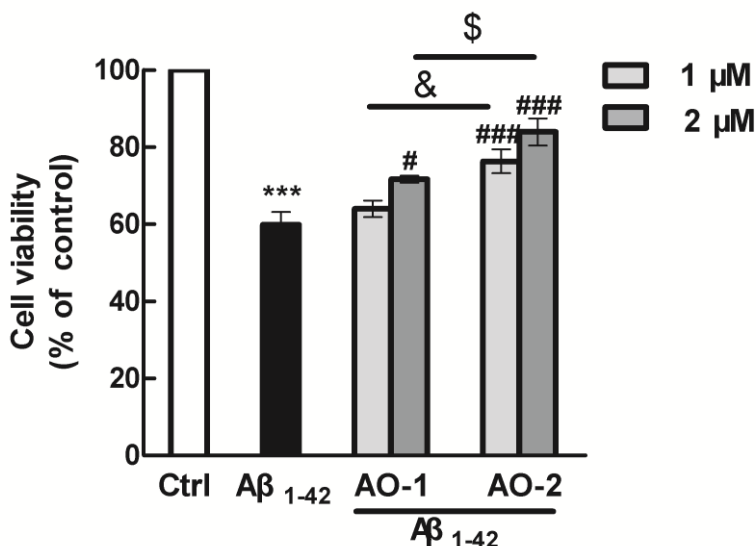


Fig 4. AO-1 and AO-2 protected against A $\beta$ <sub>1-42</sub>-induced neurotoxicity in SHSY-5Y cells. SHSY-5Y cells were pretreated with AO-1 or AO-2 (1  $\mu$ M and 2  $\mu$ M) or DMSO for 2 h, followed by 4  $\mu$ M A $\beta$ <sub>1-42</sub> together with AO-1, AO-2 or DMSO for 24 h. The cell viability levels were determined by MTT assay and normalized to cell viability of control cells treated with DMSO (100%). The statistical analyses were performed with one-way ANOVA with Bonferroni's Multiple Comparison Test.

\*\*\*P<0.001, A $\beta$ <sub>1-42</sub> vs. control group; #P<0.05, 2  $\mu$ M AO-1 vs. A $\beta$ <sub>1-42</sub>-treated group, ###P<0.0001, 1  $\mu$ M and 2  $\mu$ M AO-2 vs. A $\beta$ <sub>1-42</sub>-treated group, &P<0.05, 1  $\mu$ M AO-1 vs. 1  $\mu$ M AO-2, \$P<0.05, 2  $\mu$ M AO-1 vs. 2  $\mu$ M AO-2. Three independent experiments in each group were performed.

## 4. Conclusion

Alzheimer's disease is the most common cause of dementia in the elderly. As the treatment and nursing care of AD are worldwide problems, it is urgent to discover effective drugs for curing AD. Neuroprotection against A $\beta$ -induced cytotoxicity was considered as a beneficial response against AD. Previous studies demonstrate that AO-1 have protective effects in neurons against A $\beta$  induced damage. To discover more effective compounds, we compare the effects of AO-2 and AO-1 on A $\beta$ <sub>1-42</sub>-induced cytotoxicity in SHSY-5Y cells. AO-2 is a more active than AO-1 and is thus more suitable for further neuroprotective studies in AD models.

## Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (Grant nos. 81422012 and 31471046), the Program for New Century Excellent Talents in University of China, and the Special Support (Te Zhi) Program of Guangdong Province, China.

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