Explore the optimal concentration of ADDLs to induce neuron damage in AD model

Xiang He 1,2,a,†, Tian Meng 1,2,b,†, Qihao Zhang 1,2,c,* , Yadong Huang 1,2,c,*

1College of Life Science and Technology, Jinan University, Guangzhou 510632, China
2Institute of Biomedicine, Jinan University, Guangzhou 510632, China
† Co-first authors with equal contribution
*Corresponding Author: Qihao Zhang, Yadong Huang; Phone: +86-20-8522601
a57445292@qq.com, b417019323@qq.com, tqhzhang@jnu.edu.cn, tydhuang@jnu.edu.cn

Abstract
Alzheimer’s disease is a fatal neurodegenerative disease whose principal clinical symptoms are cognitive ability and memory loss. The main pathological features of Alzheimer’s disease are the formation of senile plaques in the brain, loss of neurons and synapses, tangles of neurofibrillary abnormal accumulation and reduction of cholinergic neurotransmitter. Aβ is used to be neurotoxin in AD model in vitro. However, Aβ-derived ligand dispersion (ADDLs) is dispersed throughout the brain and not aggregated into plaques soluble oligomers, whose neurotoxic is far stronger than the Aβ. To explore the optimal concentration of ADDLs to induce neuron damage in AD model, primary cortical neurons were isolated from one day old SD rats then cells were treated with ADDLs ( 125 nM, 250 nM, 500 nM and 1000 nM ) for 24 hrs in the 6th day. MTT results showed that ADDLs at concentration of 250 nM, 500 nM and 1000 nM reduced cortical neurons to 66.3%, 65.9% and 58.7% respectively. Microtubule-associated protein 2 (MAP2) takes part in neuronal morphogenesis. Immunofluorescence results showed that neurite in MAP2 positive neurons appeared beaded lesions, retracted and tapered in dose-effect relationship after the treatment of ADDLs. Based on above results, 250 nM ADDLs is the optimal concentration to induce neuron damage in AD model.

Keywords
Alzheimer’s disease; Aβ-derived ligand dispersion (ADDLs); Microtubule-associated protein 2 (MAP2).

1. Introduction
Alzheimer’s disease (AD) is a fatal neurodegenerative disease[1]. The Aβ theory believed that[2, 3] the deposition of Aβ caused the formation of senile plaques in brain, lead to the loss of synapses and neuronal damage, increased the intracellular oxygen free radicals. Aβ can damage neurons, induced apoptosis and loss of synapse in the classic model of AD in vitro [4]. Aβ-derived ligand dispersion (ADDLs) is a dispersed throughout the brain and not aggregated into plaques soluble oligomers, whose neurotoxic is far stronger than the Aβ. Low doses of ADDLs could significantly reduce cell viability of cortical and hippocampal neurons and cause cell death[5]. However, the optimal concentration of ADDLs to induce cell damage in primary neurons has not been reported. In this study, primary cortical neurons were isolated and treated with ADDLs to explore the optimal concentration of ADDLs in AD model in vitro.

2. Experimental detail
2.1 Materials
B27 supplemented, neurobasal medium were purchased from gibco. Aβ1-42 peptide was purchased from millipore. Anti-Oligomer antibody (A11) was purchased from invitrogen. Anti-MAP2 antibody

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was purchased from sigma and one day old SD rats were purchased from University of Traditional Chinese Medicine Experimental Animal Center.

### 2.2 Reagent formulation

#### 1. PBS buffer

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Ultra-pure water volume to 2000 mL

#### 2. Paraformaldehyde

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Ultra-pure water volume to 1800 mL

#### 3. D-HANKS

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<td>Na2HPO4.12H20</td>
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Ultra-pure water volume to 1000 mL

### 2.3 Experimental Procedure

A: Preparation of ADDLs

① The Aβ1-42 peptide was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol to 1 mM.
① The solvent was evaporated and the peptide was stored on a dried film at −80 °C.
② Dried film was resuspended in DMSO to a final concentration of 5 mM, vortexed thoroughly, and sonicated for 10 min.
③ The resulting solution was diluted with ice-cooled, phenol red-free Ham’s F12 medium to 100 μM and centrifuged at 2,500 rpm for 5 min, the supernatant was removed to a new EP tube and incubated stored at 4 °C for 24 hrs to allow Aβ oligomers to form.
④ The oligomer solution was centrifuged at 4 °C 14,000 g for 10 min, and the supernatant containing soluble Aβ oligomers was collected.
⑤ Protein concentration was determined by Pierce BCA protein assay kit.
⑥ 16% Tricine- SDS-PAGE and immunoblot were used to identify Aβ oligomers.

B: MTT
① After cortical neurons of SD neonatal rats were treated with ADDLs for 24 hrs, 10 μl 5 mg/ml of MTT was added in each well then samples were incubated for 4 hrs in dark at 37 °C.
② Siphoning off the liquid in each well, 150 μl of DMSO was added respectively in dark at 37 °C.
③ Cortical neurons were placed in microplate reader to detect absorbance in 490 nm wavelength.

C: Immunofluorescence (MAP2)
① Cortical neurons of SD neonatal rats were cultured in confocal petri dish for 7 days. Then the medium was sucked, washed with PBS one time;
② With incubation of 4% paraformaldehyde for 10 min at room temperature, cells were washed with PBS three times;
③ Cells were treated at room temperature with 0.1% of Triton X-100 for 10 min, then washed three times with PBS;
④ With 1% BSA blocking for 1 hr at room temperature;
⑤ Incubated protein MAP2 antibody overnight at 4 °C, then washed with PBS three times;
⑥ Cy3-labeled goat anti-rabbit secondary antibody was incubated in dark for 1 hr. Then washed with PBS five times;
⑦ Siphoning off the liquid in the dish, cells were covered with anti-fluorescence quenching liquid and observed in a laser scanning confocal microscopy.

2.4 Statistical analysis
All data are presented as mean±SD and statistically significant were determined by one-way ANOVA. p value less than 0.05 was considered statistically significant.
3. Results and discussion

Anti-oligomer antibody (A11) could identify any aggregate state of Aβ. As shown in Figure 1, Aβ was detected as a monomer form, which is observed at a molecular weight of 4 kDa by western blotting. ADDLs exhibited multiple forms of Aβ including monomer (4kDa), dimer (8kDa), tetramer (16kDa) and a few larger oligomers (105kDa). This result suggested that ADDLs were successfully prepared.

Cortical neurons were incubated with different concentrations ADDLs (125 nM, 250 nM, 500 nM, 1000 nM) for 24 hrs, MTT assay was used to detect neurotoxic effects of ADDLs. As shown in Figure 2, ADDLs at 250 nM, 500 nM, 1000 nM could reduce cortical neurons viability to 66.3%, 65.9% and 58.7% respectively. All of them were statistically significant compared to the control group (p < 0.001).

Microtubule-associated protein 2 (MAP2) belongs to a family of heat stable MAPs, which takes part in neuronal morphogenesis, maintaining cellular architecture and internal organization, cell division and cellular processes[6]. It has been reported that in the brains of Alzheimer’s diseases, the levels of MAP2 are usually decreased[7]. As shown in Figure 3, neurite in MAP2 positive neurons appeared beaded lesions, retracted and tapered in dose-effect relationship after the treatment of ADDLs.

Figure 2 Cell viability of cortical neurons with treatment of different concentrations of ADDLs. n=5. *p < 0.05, ** p < 0.01, *** p < 0.001 compared to the control group.

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Figure 3 Morphous of neurite in MAP2 positive neurons treated with different concentrations of ADDLs (400×). C: control, As125: 125 nM ADDLs, As250: 250 nM ADDLs, As500: 500 nM ADDLs, As1000: 1000 nM ADDLs.
4. Conclusion

In this study, we found that ADDLs at concentrations of 250 nM, 500 nM and 1000 nM reduced cortical neurons to 66.3%, 65.9% and 58.7% respectively. And the results of immunofluorescence showed that neurite in MAP2 positive neurons appeared beaded lesions, retracted and tapered in dose-effect relationship after the treatment of ADDLs. Based on above results, 250 nM ADDLs is the optimal concentration to induce neuron damage in AD model, which will provides a new way of AD research in cortical neurons in vitro.

Acknowledgements

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Reference