Effect of Tiantai No.1 (天泰1号) on β-Amyloid-induced Neurotoxicity and NF-κB and cAMP Responsive Element-binding Protein

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ABSTRACT  Objectives: To investigate the effect and molecular mechanism of Tiantai No.1 (天泰1号), a compound Chinese herbal preparation, for the prevention and reduction of neurotoxicity induced by beta-amyloid peptides (Abeta) in vitro and its effects on nuclear factor-κ B (NF-κ B) and cAMP responsive element-binding protein (CREB) pathways using the gene transfection technique. Methods: B104 neuronal cells were used to examine the effects of Tiantai No.1 on lowering the neurotoxicity induced by Abeta. The cells were co-treated with Tiantai No.1 and beta-amyloid peptide 1-40 (Aβ 1-40, 10 μmol/L) for 48 h or post-treated with Tiantai No.1 for 48 h after the cells were exposed to beta-amyloid peptides 25-35 (Aβ 25-35) for 8 h. In gene transfection assays, cells were treated with Tiantai No.1 at 50 μg/mL and 150 μg/mL for 5 days or co-treated with Tiantai No.1 and Aβ 1-40 (5 μmol/L) for 3 days after electroporation for the evaluation of NF-κ B and CREB expression. Results: Pre-treating and co-treating B104 neuronal cells with Tiantai No.1 lowered the neurotoxicity induced by Abeta, and post-treating with Tiantai No.1 reduced or blocked B104 neuronal apoptotic death induced by Abeta (P<0.05, P<0.01). With a dose-dependent relationship, the same treatments increased the expression of NF-κ B or CREB in B104 neuronal cells (P<0.05, P<0.01). Meanwhile, Tiantai No.1 reduced Aβ 40 induced inhibition on NF-κ B expression (P<0.01). Conclusions: Tiantai No.1 can protect neurons against the neurotoxicity induced by Abeta. The neuroprotective mechanisms may be associated with the activation of NF-κ B and cAMP cellular signal pathways.

KEY WORDS  Alzheimer’s disease, beta-amyloid peptide, apoptosis, nuclear factor-κ B, cAMP responsive element-binding protein, Tiantai No.1

Alzheimer’s disease (AD) is the most prevalent age-associated neurodegenerative disease. There is no proven way for the prevention and treatment of AD. The etiology of AD is still unclear, but plenty of evidence demonstrates that beta-amyloid peptides (Abeta) may play a key role in the etiology and/or the progression of AD.

In the brains of AD patients, the characteristic pathological feature is the deposition of Abeta in senile plaques. Neurotoxic and apoptotic effects of Abeta activity-related fragments Abeta 25-35 and Abeta 1-40 have been demonstrated in different regions involved in the pathogenesis of AD. Substantial evidence suggests that the accumulation of beta-amyloid-derived peptides may contribute to the etiology and/or the progression of AD.

Tiantai No.1 (天泰1号) is a prepared herbal extract product based on many years of clinical experiences and pharmacological studies in AD. The present studies are designed to determine the effects of Tiantai No.1 to reduce or prevent the neurotoxicity induced by Abeta, and to elucidate the possible mechanisms of cellular and molecular pathways involving transcription factors nuclear factor-κ B (NF-κ B) and cAMP responsive element-binding protein (CREB).

METHODS

Materials

Obtained from Shenzhen Institute of Integrated Traditional and Western Medicine in China, Tiantai No.1 is a compound herbal product consisting mainly of the following active ingredients:...
of *Gastrodia elata* Bl., *Panax ginseng* C. A. Meyer, and *Folium Gindgo* in a ratio of 2:2:1 and manufactured according to a well-defined procedure, each gram containing 20 g of crude drugs. The drug product was dissolved in the injection water and confected to a 10% concentration, and then deposited in a 4 °C refrigerator after filtration and sterilization.

Beta-amyloid peptides (Abeta1-40, Lot. 00101409; Abeta25-35, Lot. 0314014) were purchased from Quality Controlled Biochemicals, Inc (QCB); pNF-κ B-Luc, pCRE-Luc and pEGFP-N2 plasmids were obtained from clontech; luciferase assay substrate (Lot. 5509701) and luciferase assay buffer (Lot. 5576501) obtained from Promega Corporation; protein assay dye reagent concentrate (Lot. 51887A) was obtained from Bio-Rad Laboratories; isopropanol anhydrous (Lot. 115H4363) was obtained from Sigma; LIVE/DEAD Viability/Cytotoxicity Kit (Lot. 3224) was obtained from Molecular Probes; Dulbecco's modified eagle medium (DMEM) and other cell culture materials were obtained from GIBCO BRL (Life Technologies); B104 cell line was derived from rat brain neuroblastoma and obtained from the Brain Institute of UCLA in the USA.

**Cell Culturing**

*B104* cells were maintained in DMEM supplemented with 5% fetal calf serum, 100 U/mL penicillin and 100 U/mL streptomycin antibiotics. Monolayer cultures were maintained in plastic flasks in a 95% air, 5% CO₂ humidified atmosphere at 37 °C. The culture medium was changed once every 48 h.

**Tiantai No.1 against the Neurotoxicity of Abeta1-40**

The test was divided into 5 groups: the normal control, the Abeta1-40 model control, Abeta1-40 plus low-, middle-, and high- dosage Tiantai No.1 (50, 100, 200 μg/mL) groups. B104 cells were plated with cell density of 1.25 × 10^5 cells per well in 24-well plates, and then incubated in DMEM for 48 h. Then the medium was replaced as fetal calf serum-free and included Abeta1-40 (25 μmol/L). After 8 h, the medium was removed and the cells were gently washed once by D-PBS. Then the cells were treated with Tiantai No.1 and Abeta1-40 for 48 h, and the cell survival was determined.

**Fluorescence Staining and Observation for Apoptotic Cells**

Cell apoptosis was determined using the calcein AM/ethidium homodimer-1 technique (LIVE/DEAD Viability/Cytotoxicity Kit). After the drug treatment, the medium was removed and the cells were washed gently once with 1× D-PBS. Then a proper volume (50 μL per well of 24-well plate, 100 μL per well of 12-well plate or 150 μL per well of 6-well plate) of the combined working staining solution (2 mmol/L calcein AM and 4 mmol/L ethidium homodimer-1) was added directly to the cells, and the cells were incubated at room temperature for 30 min. The labeled live and dead cells were immediately observed and photographed using a Zeiss fluorescence microscope (Zeiss Axiovert 135M, Mercury Lamp, Carl Zeis HBO 100W/Z) with 485 nm and 590 nm filters.

**Live-cell Protein Determination**

After the drug treatment, the cells were washed three times with D-PBS to remove dead cells. Then 50 μL of lysis buffer (commercial kit ApoAlert CPP32, Clontech Laboratories) was added to each well of the 24-well plate. The cells were incubated at room temperature for 10 min. After incubation the cellular lysates were centrifuged at 12 000 r/min for 3 min, then the supernatant was extracted and the protein content was determined with a commercial protein assay kit (Bradford method, BioRad Laboratories) and 3000-Smartspect (Bio-RAD).

**Genes Transfection and Luciferase Assay**

Transfection of B104 cells with pNF-κ B and pCRE was performed using electroporation via the Four Parameter PulseAgile TM Electroporation System