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Notoginsenoside R1 attenuates amyloid-β-induced damage in neurons by inhibiting reactive oxygen species and modulating MAPK activation

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ABSTRACT

Progressive accumulation of amyloid-β (Aβ) is a pathological hallmark of Alzheimer's disease (AD). Aβ increases free radical production in neuronal cells, leading to oxidative stress and cell death. An intervention that would reduce Aβ-related neurotoxicity through free radical reduction could advance the treatment of AD. Notoginsenoside R1 (NR1), the major and most active ingredient in the herb *Panax notoginseng*, can reduce reactive oxygen species and confer some neuroprotective effects. Here, NR1 was applied in a cell-based model of Alzheimer's disease. Cell viability, cell death, reactive oxygen species generation, and mitochondrial membrane potential were assessed in cultured PC12 neuronal cells incubated with $A\beta_{25-35}$. In this model, Aβ was neurotoxic and induced necrosis and apoptosis; however, NR1 significantly counteracted the effects of Aβ by increasing cell viability, reducing oxidative damage (including apoptosis), restoring mitochondrial membrane potential, and suppressing stress-activated MAPK signaling pathways. These results promise a great potential agent for Alzheimer's disease and other Aβ pathology-related neuronal degenerative disease.

1. Introduction

Alzheimer's disease (AD) is the most common human age-related neurodegenerative disorder. AD pathology is characterized by deposition of extracellular senile plaques and intracellular neurofibrillary tangles and selective loss of synapses and neurons [1]. A common feature of AD is the accumulation of amyloid-β (Aβ) [2], 39–43 amino acid peptides produced by cleavage of the amyloid precursor protein by β- and γ-secretases [3].

Aβ peptides aggregate to form fibrillar deposits that are the principal component of senile plaques. Numerous studies have demonstrated that fibrillar Aβ aggregates, but not soluble Aβ, are neurotoxic [4]. Therefore, Aβ aggregates are thought to be the central molecules in the pathological development of AD. Aβ-induced toxicity involves oxidative stress, inflammation, and perturbation of calcium homeostasis [5]. In addition, both necrotic and apoptotic processes occur in primary neurons and neuronal cell lines after exposure to micromolar concentrations of Aβ [6,7]. However, the underlying mechanisms of toxicity and the neuronal cellular signaling cascades activated by Aβ are not fully understood.

Between its two predominant forms, $A\beta_{1-42}$ is dominant in senile plaques and has stronger aggregation propensity and increased toxicity than $A\beta_{1-40}$. $A\beta_{25-35}$ exerts neurotoxic effects similar to those produced by parent $A\beta_{1-40/42}$ peptides, such as learning and memory impairment, neuronal apoptosis, and oxidative stress; therefore, $A\beta_{25-35}$ is used to establish AD models for studying the neurotoxic properties of Aβ and for drug screening [5]. Because memory loss and cognitive dysfunction are the main clinical symptoms of AD patients, treatment and prevention of AD have stimulated the search for novel agents that can confer protection against learning and memory impairment.

Notoginsenoside R1 (NR1) (Fig. 1) is the main compound in *Panax notoginseng*, an herbal medicine widely used in Asia for the treatment of cardiovascular disease and cerebral vascular diseases [8]. Several recent studies have indicated that NR1 can attenuate the risk of human cardiovascular and cerebrovascular diseases through antioxidant, anti-inflammatory, anti-angiogenic, anti-apoptosis, and other biological activities [9–11]. Furthermore, More and more researches have shown that NR1 has remarkable neuroprotective effect, it can improve ischemia–reperfusion injury in vivo and prevent various damages (oxidative, glutamate, LPS, Aβ, etc.) of nerve cells (primary neurons, neurogliocytes, PC12 cells, etc.) in vitro [12–16]. NR1 has also been demonstrated that it could be absorbed into the bloodstream by pharmacokinetic studies [17,18], so it is becoming a new potential drug

Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; Aβ, amyloid-β; DA, diacetate; DCF, 2′,7′-dichlorofluorescein; DMSO, dimethyl sulfoxide; ERK 1/2, extracellular signal-regulated kinases 1/2; H2DCF, reduced DCF; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; NR1, notoginsenoside R1; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species.

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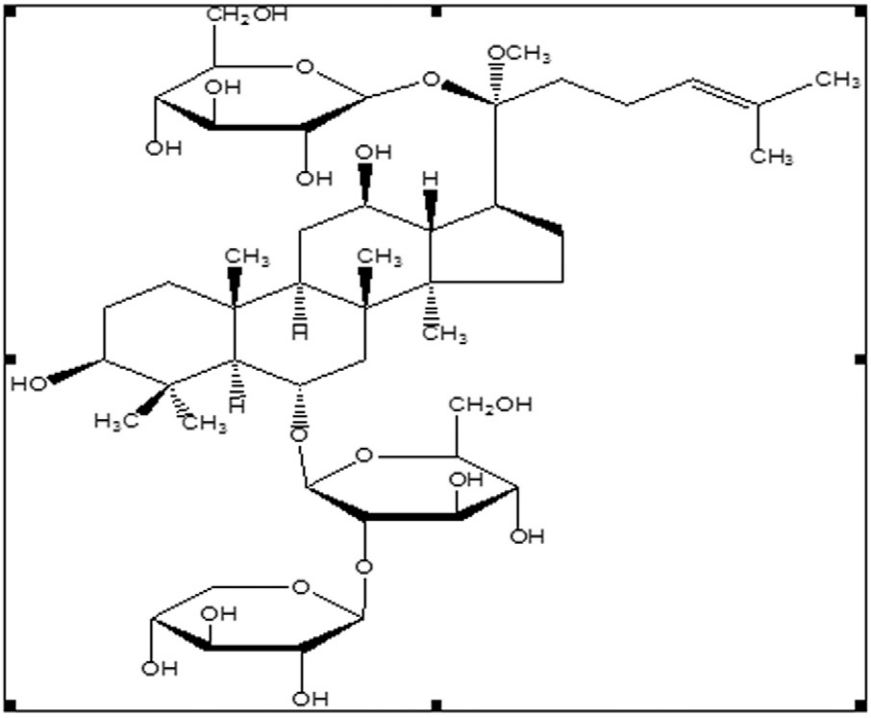

Fig. 1. Molecular structure of notoginsenoside R1.

candidate. Here, we mainly investigated the protective effects of NR1 and their possible mechanisms in PC12 cells exposed to $A\beta_{25-35}$.

2. Materials and methods

2.1. Materials

$A\beta_{25-35}$, $A\beta_{1-42}$, Fluorescent dyes Hoechst 33342, propidium iodide (PI), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and rhodamine 123 were purchased from Sigma (St. Louis, Missouri, USA). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA), Annexin V/fluorescein isothiocyanate/propidium iodide (FITC/PI) apoptosis kit, and terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end-labeling (TUNEL) kit were purchased from Invitrogen (Carlsbad, California, USA). Dulbecco's modified Eagle medium (DMEM), Neurobasal™ medium, B-27 serum-free supplement, fetal bovine serum (FBS), and penicillin–streptomycin were purchased from Gibco BRL (Life Technologies, Paisley, Scotland). Anti-ERK1/2, anti-p-ERK1/2, anti-p38, anti-p-p38, anti-JNK, anti-p-JNK, anti-caspase 3, anti-BAX, anti-BCL2, and anti-β-actin antibodies were obtained from Santa Cruz Biotechnology (CA, USA). Notoginsenoside R1 (purity ≥ 98%) was obtained from the Shanghai Winherb Medical S & T Development Co. Ltd. (Shanghai, China). It was dissolved in 0.1% acetic acid at a concentration of 1 mM as stock solutions, the stock was diluted to the desired final concentrations in treatment medium prior to use.

2.2. Preparation of aggregated Aβ

$A\beta_{25-35}$ and $A\beta_{1-42}$ was dissolved in deionized distilled water at a concentration of 1 mM and incubated at 37 °C for 4 days to induce aggregation separately [19]. After aggregation, the solution was stored at −20 °C until use.

2.3. Cell culture

Rat PC12 pheochromocytoma cells were obtained from the Institute of Basic Medical Sciences at the Chinese Academy of Medical Sciences. Cells were cultured in DMEM supplemented with 5% horse serum, 10% FBS, and 100 U/mL of penicillin–streptomycin at 37 °C with a 5% CO_2 atmosphere in a humidified incubator. PC12 cells were subcultured about twice a week and split 1:4 when the culture was 80–90% confluent.

Primary cultured cortical neurons were obtained from embryonic (E18d) Sprague–Dawley rat fetuses. Briefly, the brains were removed and the cortices were dissected out. The tissues were cut into fragments, and incubated in 0.2% trypsin for 20 min in 37 °C. Then, DMEM medium containing 10% FBS was added for trypsin inactivation, and the tissues were dissociated by mild mechanical trituration. About 1×10^5 cells/mL were plated onto poly-D-lysine-coated 96-well plates for further culture. After 4 h, the DMEM medium was changed into Neurobasal™ medium supplemented with 2% B-27 serum-free supplement and 0.5 mM L-glutamine in a humidified incubator containing 95% air and 5% CO_2 at 37 °C. Primary neuronal cultures were maintained for 6–7 days before experiments.

2.4. MTT assay

The MTT assay measures cell proliferation rate and cell viability by chromogenic changes of MTT dye, which is reduced to a blue-violet formazan by mitochondrial succinate dehydrogenase in viable cells. Approximately 10,000 cells/well were seeded in a flat 96-well microplate in triplicate. After 36 h, cells were then incubated with freshly prepared $A\beta_{25-35}$/$A\beta_{1-42}$ (2.5–40 μM) for 24 h, cell viability and half maximal inhibitory concentrations (IC_{50}) were determined by MTT. To determine the protective effects of NR1 on Aβ-induced neurotoxicity, both PC12 cells and primary neurons were pretreated with different concentrations of NR1 (1–100 μM) for 24 h. Cells were then incubated with $A\beta_{25-35}$ (20 μM) or $A\beta_{1-42}$ (10 μM) for an additional 24 h. MTT solution (20 μL of 5 mg/mL stock solution in PBS per well) was added for 3 h at 37 °C, medium was aspirated, and 150 μL DMSO was added. The absorbance at 570 nm was determined using a microplate reader (SpectraFluor; Tecan, Sunrise, Austria). Experiments were repeated in triplicate and data were expressed as percentages of control.