Biochemical and Biophysical Research Communications 470 (2016) 798–803

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Neurotrophic activity of jiadifenolide on neuronal precursor cells derived from human induced pluripotent stem cells

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ARTICLE INFO

Article history:
Received 22 December 2015
Accepted 15 January 2016
Available online 22 January 2016

Keywords:
Neurotrophin
Jiadifenolide
hiPSC
Neurite outgrowth
MAP2
PSD95

ABSTRACT

Although jiadifenolide has been reported to neurotrophin-like activity in primary cultured rat cortical neurons, it is unknown on that of activity in human neurons. Thus, we aimed to assess neurotrophin-like activity by jiadifenolide in human neuronal cells. We analyzed neuronal precursor cells derived from human induced pluripotent stem cells for microtuble-associated-protein-2 expression by immunofluorescence and western blot, following jiadifenolide treatment. Jiadifenolide promoted dendrite outgrowth, facilitated growth, and prevented death in neuronal cells derived from human induced pluripotent stem cells. Interestingly, jiadifenolide also increased postsynaptic density-95 protein expression suggesting that jiadifenolide promotes neuronal maturation and post-synaptic formation. We demonstrate for the first time that jiadifenolide exhibits neurotrophic effects on human neuronal precursor cells.

1. Introduction

Neurotrophic factors (neurotrophins) such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) have been reported to prevent neuronal degeneration, promote neurite regeneration and neuronal survival, and enhance synaptic plasticity [1,2]. Thus, neurotrophins have been investigated for therapeutic application in neurological diseases including Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis (ALS) that show neurodegenerative, behavioral, and psychiatric symptoms [3,4]. However, although neurotrophins act largely within the brain, these proteins have high molecular weights, limiting serum-stability and blood-brain-barrier passage [5–7]. Moreover, clinical trials of NGF and BDNF have failed [8]. Thus, novel small-molecule neurotrophin-like compounds are needed, which lack such pharmacological disadvantages.

Jiadifenolide (Fig. 1), a seco-prezizaane-type sesquiterpenoid, was isolated from the pericarps of *Illicium jiadifengpi* collected in Southwestern China [9], and was chemically synthesized [10]. Jiadifenolide has very unique chemical structure (Fig. 1) [9, 10]. Jiadifenolide and derivatives have been reported to promote neurite outgrowth in primary cultured rat cortical neurons [9] and enhance NGF activity, thereby facilitating differentiation of PC12 cells (a rat adrenal pheochromocytoma cell line) [11]. Thus, previous studies suggest these compounds have neurotrophin-like effects in rat neurons. However, owing to species differences, there have been limitations in accurately predicting the clinical activities and adverse effects of jiadifenolide in human neurons. Therefore, prior to conducting clinical trials, an investigation of the neurotrophic effects of jiadifenolide on human neurons is necessary.

2. Materials and methods

2.1. Preparation of jiadifenolide

Jiadifenolide (Fig. 1) as chemically synthesized from neomajucin isolated from the pericarps of *Illicium jiadifengpi*, and purified as previously reported [9]. We dissolved synthetic jiadifenolide in dimethyl sulfoxide (DMSO) at a concentration of 10 mM for stock solutions.

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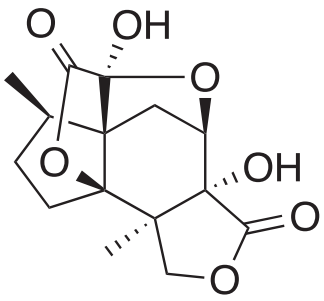
http://dx.doi.org/10.1016/j.bbrc.2016.01.092



jiadifenolide

Fig. 1. Chemical structure of jiadifenolide.

2.2. Culture of human induced pluripotent stem cells (hiPSCs)

Human induced pluripotent stem cells (hiPSCs) (line 201B7; Cell No. HPS0063) [12], were purchased from a cell bank (Riken Bioresource Center, Ibaraki, Japan). Cells were maintained on a mitomycin-C (Kyowa Hakko Kirin, Tokyo, Japan)-treated SNL 76/7 feeder-cell (DS Pharma Biomedical, Osaka, Japan) layer at 37 °C and 5% CO_2. Cells were grown in a primate embryonic stem cell medium (ReproCELL, Kanagawa, Japan), supplemented with 4 ng/mL basic fibroblast growth factor (Wako, Osaka, Japan) and 50 U/mL penicillin with 50 μg/mL streptomycin (P/S; Life Technologies, CA, USA).

2.3. Induction of neuronal precursor cell differentiation

Differentiation of neuronal precursor cells from hiPSCs was induced by serum-free culture of embryoid body-like aggregates, quick (SFEBq) method [13,14] (Fig. 2, stages I and II). Briefly, hiPSCs cultured on a mitomycin C-treated SNL feeder-cell layer were treated with 10 μM Y-27632, Rho-associated coiled-coil forming kinase/Rho-associated kinase inhibitor, (Wako, Osaka, Japan), and were dissociated into single cells using TrypLE™ Select solution (Life Technologies) after removing feeder cells using CTK solution (2.5% trypsin [Life Technologies], 1 mg/mL collagenase IV [Life Technologies], 0.1 M $CaCl_2$ and 20% knockout serum replacement [KSR; Life Technologies] in H_2O). The hiPSCs were aggregated in a low-binding, U-bottomed, 96-well plate at 37 °C and 5% CO_2 and 5% DFK medium (5% KSR [Life Technologies], 100 μM modified Eagle medium, nonessential amino acids solution [Life Technologies], 2 mM L-glutamine, 110 μM 2-mercaptoethanol [Life Technologies], and P/S [Life Technologies] in glucose-Dulbecco's modified Eagle medium (DMEM)/F12 medium [Life Technologies], supplemented with 10 μM Y-27632, 2 μM dorsomorphin (Calbiochem, CA, USA) and 10 μM SB431542, transforming growth factor β1 superfamily activin receptor-like kinase inhibitor, (Stemgent, MA, USA) for embryoid-body (EB) formation and neuronal induction (Fig. 2, stage I). Half of media in wells was changed with 5% DFK medium at either 3 or 4 days of incubation. After 10–12 days of incubation, EBs were transferred onto a Matrigel matrix (Corning, MA, USA)-coated 6-well plate and cultured for 16 days at 37 °C and 5% CO_2 with 5% DFK medium supplemented with 1% N2 supplement (Life Technologies), 2 μM dorsomorphin, and 10 μM SB431542. Neuronal precursor cells were removed from EB cores [14] (Fig. 2, stage II).

2.4. Maturation of neuronal cells

The procedure used to induce neuronal maturation was performed as indicated in Fig. 2, stage III. Neuronal precursor cells removed from EB cores were dissociated into single cells with an accutase (Life Technologies), and were then cultured on both 0.01% poly-L-ornithine (Sigma, MO, USA) and 3.3 μg/mL laminin (Sigma)-coated 24-well plates at 37 °C and 5% CO_2, with neurobasal medium (Life Technologies), supplemented with 2% B-27 supplement (Life Technologies) and P/S. Depending on treatment conditions, media also contained one of the following: (i) 0.25% DMSO (as a negative control); (ii) neurotrophins (as a positive control; 10 ng/mL recombinant human BDNF [Wako], 10 ng/mL recombinant human glial-cell derived neurotrophic factor [GDNF; Wako], and 10 ng/mL recombinant human NT-3 [Wako] [14]); or (iii) 1, 5, 10, or 25 μM jiadifenolide (Fig. 2, stage III). Media in wells was changed at either 3 or 4 days of incubation.

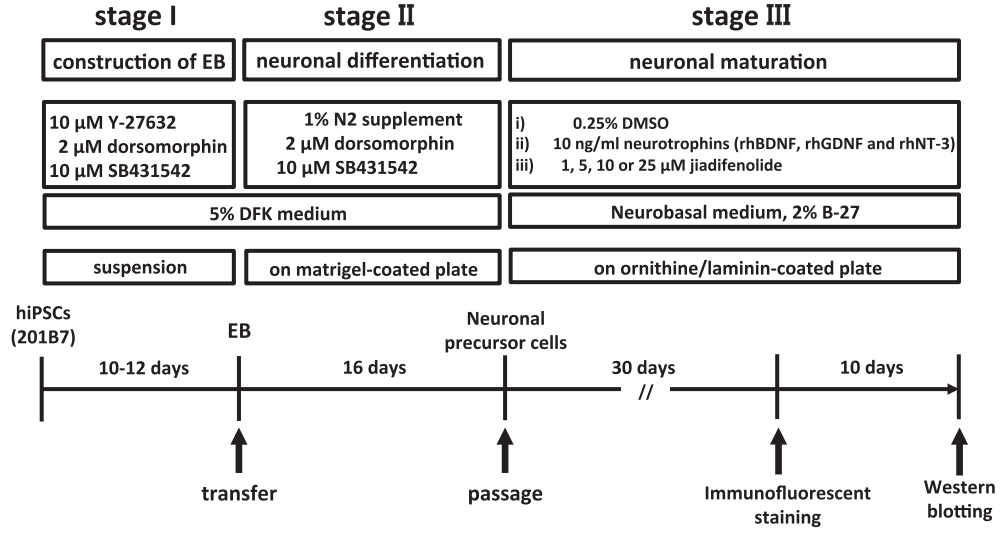


Fig. 2. Experimental procedure for neuronal cell differentiation and maturation from hiPSCs. To evaluate the neurotrophic effects of jiadifenolide on human neurons, Jiadifenolide was added at stage III. Immunofluorescent staining and western blots were performed on neuronal precursor cells derived from hiPSCs treated with (i), (ii), or (iii), at 30 or 40 days post-incubation, respectively. Additional details are described in Materials and Methods.