Neurotoxicology 67 (2018) 259–269

Contents lists available at ScienceDirect

Neurotoxicology

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

Full Length Article

The indole compound NC009-1 inhibits aggregation and promotes neurite outgrowth through enhancement of HSPB1 in SCA17 cells and ameliorates the behavioral deficits in SCA17 mice

Chiung-Mei Chen[a], Wan-Ling Chen[a], Chen-Ting Hung[b], Te-Hsien Lin[b], Chih-Ying Chao[a], Chih-Hsin Lin[a], Yih-Ru Wu[a], Kuo-Hsuan Chang[a], Ching-Fa Yao[c], Guey-Jen Lee-Chen[b,*], Ming-Tsan Su[b,*], Hsiu Mei Hsieh-Li[b,*]

[a] Department of Neurology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taipei 10507, Taiwan
[b] Department of Life Science, National Taiwan Normal University, Taipei 11677, Taiwan
[c] Department of Chemistry, National Taiwan Normal University, Taipei 11677, Taiwan

ARTICLE INFO

Keywords:
Spinocerebellar ataxia type 17
TATA box binding protein
PolyQ aggregation
Indole compound
HSPB1
Therapeutics

ABSTRACT

Spinocerebellar ataxia type 17 (SCA17) is caused by the expansion of translated CAG repeat in the TATA box binding protein (*TBP*) gene encoding a long polyglutamine (polyQ) tract in the TBP protein, which leads to intracellular accumulation of aggregated TBP and cell death. The molecular chaperones act in preventing protein aggregation to ameliorate downstream harmful events. In this study, we used Tet-On cells with inducible SCA17 TBP/Q_{79}-GFP expression to test five in-house NC009 indole compounds for neuroprotection. We found that both aggregation and polyQ-induced reactive oxygen species can be significantly prohibited by the tested NC009 compounds in Tet-On TBP/Q_{79} 293 cells. Among the five indole compounds, NC009-1 up-regulated expression of heat shock protein family B (small) member 1 (HSPB1) chaperone to reduce polyQ aggregation and promote neurite outgrowth in neuronal differentiated TBP/Q_{79} SH-SY5Y cells. The increased HSPB1 thus ameliorated the increased BH3 interacting domain death agonist (BID), cytochrome c (CYCS) release, and caspase 3 (CASP3) activation which result in apoptosis. Knock down of HSPB1 attenuated the effects of NC009-1 on TBP/Q_{79} SH-SY5Y cells, suggesting that HSPB1 might be one of the major pathways involved for NC009-1 effects. NC009-1 further reduced polyQ aggregation in Purkinje cells and ameliorated behavioral deficits in SCA17 TBP/Q_{109} transgenic mice. Our results suggest that NC009-1 has a neuroprotective effect on SCA17 cell and mouse models to support its therapeutic potential in SCA17 treatment.

1. Introduction

Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenrative disease, characterized by progressive ataxia and/or declined cognitive function, psychiatric symptoms, seizures, pyramidal and extrapyramidal features such as spasticity, dystonia, chorea, or parkinsonism (Lasek et al., 2006). SCA17 is caused by an expanded allele containing repeats longer than 43 in the TATA-box binding protein (*TBP*) gene, a transcription initiation factor (Koide et al., 1999). The expanded allele encodes a polyglutamine (polyQ) tract. SCA17 is one of hereditary neurodegenerative diseases caused by expanded polyQ tracts (Everett and Wood, 2004).

The pathogenic processes of SCA17 have been explored and transcriptional dysregulation is one of the main pathogenic mechanisms (Yang et al., 2016). It have been shown that mutant TBP with expanded polyQ interacts aberrantly with several specific transcription factors such as Sp1 (Shah et al., 2009), RBP-J/Su(H) (Ren et al., 2011), transcription factor IIB (TFIIB) (Friedman et al., 2007), nuclear factor-Y (NFY) (Huang et al., 2011; Lee et al., 2012), X-box binding protein 1 (XBP1) (Yang et al., 2014), and MyoD1 (Huang et al., 2015) to impair the transcriptional function. Among them, Friedman and colleagues have shown that mutant TBP impaired the TFIIB function by binding more strongly to TFIIB, which would lead to a reduced level of the TFIIB-dependent gene transcription, the small heat shock protein HSPB1 (HSP27), in a transgenic (TG) mouse model of SCA17 (Friedman et al., 2007). We have also previously shown down-regulation of HSPB1 in lymphoblastoid cells of SCA17 patients (Chen et al., 2010). In addition, decreased HSPB1 has been demonstrated in other polyQ diseases including Huntington's disease (HD) (Wyttenbach et al., 2002), spinocerebellar ataxia type 3 (SCA3) (Wen et al., 2003; Chang et al., 2009)

* Corresponding authors.
E-mail addresses: t43019@ntnu.edu.tw (G.-J. Lee-Chen), mtsu@ntnu.edu.tw (M.-T. Su), hmhsieh@ntnu.edu.tw (H.M. Hsieh-Li).

https://doi.org/10.1016/j.neuro.2018.06.009
Received 1 November 2017; Received in revised form 14 June 2018; Accepted 14 June 2018
Available online 21 June 2018
0161-813X/

and spinocerebellar ataxia type 7 (SCA7) (Tsai et al., 2005). Over-expression of HSPB1 is able to rescue neurons from polyQ-induced toxicity and neurodegeneration in HD cellular (Wyttenbach et al., 2002) and SCA17 animal (Friedman et al., 2007, 2009) models. Therefore, strategies or compounds enhancing HSPB1 activity may have therapeutic effects in SCA17.

Indole is an aromatic heterocyclic compound with a six-membered benzene ring fused to a five-membered pyrrole ring (C_8H_7N). Previously indole compound indomethacin ($C_{19}H_{16}ClNO_4$) was reported to induce the expression of constitutive HSC70 (HSPA8)/inducible HSP70 (HSPA1 A) and HSP105 A heat shock proteins to suppress polyQ aggregation in a cellular model of spinal and bulbar muscular atrophy (Ishihara et al., 2004). We have also shown that indole and derivative NC001-8 ($C_{11}H_9NO$) up-regulated heat shock transcription factor HSF1 and HSC70/HSP70 chaperone expression to reduce polyQ aggregation in neuronal differentiated SCA3/SCA17 cells (Lin et al., 2014; Kung et al., 2014). As indole derivatives are possible therapeutics for polyQ-mediated diseases and an in-house C-alkylated indole compound NC009-1 ($C_{19}H_{16}N_2O_3$) enhances HSPB1 expression in ΔK280 tau_{RD}-DsRed model (Chang et al., 2017), we examined the effects of five NC009 group indole compounds (Ramesh et al., 2009) on enhancing HSPB1 expression using Tet-On 293/SH-SY5Y cells with inducible SCA17 TBP/Q_{79}-GFP expression (Lee et al., 2015; Kung et al., 2014). In addition, neuroprotective effect of NC009-1 on a previously established SCA17 TBP/Q_{109} TG mouse model (Chang et al., 2011) was explored.

2. Materials and methods

2.1. Tested indole compounds

Indole compounds NC009-1, 2, 3, 6 and 11 (Fig. 1A) were synthesized and characterized by NMR spectrum as described (Ramesh et al., 2009). No geometric isomers occur in these tested compounds.

2.2. Cell culture and cell proliferation assay

SH-SY5Y cells are often used as *in vitro* models of neuronal function and differentiation (Påhlman et al., 1984; Agholme et al., 2010). Tet-On TBP/Q_{79}-GFP 293/SH-SY5Y cells (Kung et al., 2014; Lee et al., 2015) were cultivated in DMEM medium containing 10% fetal bovine serum (FBS, Invitrogen), 5 μg/ml blasticidin and 100 μg/ml hygromycin (InvivoGen) at 37 °C incubator with 5% CO_2 atmosphere. The TBP/Q_{79}-GFP expression is induced by the addition of doxycycline which regulates the human cytomegalovirus (CMV)/$TetO_2$ promoter-controlled transgene expression.

Cell proliferation was measured based upon the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, 5×10^4 cells were plated into 48-well dishes, grown for 20 h, and treated with indole compounds (100 nM to 10 − 100 μM). After 1 day, 20 μl MTT (5 mg/ml in phosphate-buffered saline (PBS), Sigma-Aldrich) was added to the cells and incubated for 2 h. The absorbance of the insoluble purple formazan product was measured at 570 nm by a Bio-Tek μQuant Universal microplate spectrophotometer.

2.3. 293 TBP/Q_{79} aggregation assay

293 TBP/Q_{79}-GFP cells were plated into 96-well (2×10^4/well) dishes, grown for 24 h and treated with different concentrations of the NC009 compounds (0.1 nM to 10 − 100 μM), or suberoylanilide hydroxamic acid (SAHA) (0.1 μM, Cayman Chemical) as a positive control for 8 h. Then doxycycline (10 μg/ml, Sigma-Aldrich) was added for 6 days to induce TBP/Q_{79}-GFP expression. In addition, oxaliplatin (5 μM, Sigma-Aldrich) was added for aggregate accumulation through inhibition of DNA synthesis and cell cycle arrest (Flis and Spławiński, 2009). After that, the cells were stained with Hoechst 33342 (0.1 μg/ml, Sigma-Aldrich) and images of the cells were automatically obtained using a high content analysis (HCA) system (ImageXpressMICRO, Molecular Devices), with excitation/emission wavelengths at 482/536 (enhanced GFP). Aggregation was determined by Transfluor technology (Ghosh et al., 2005) based on GFP intensity. To quantify aggregation, the relative aggregation level in untreated cells is set as 100%.

2.4. Reactive oxygen species (ROS) analysis

293 TBP/Q_{79}-GFP cells were plated into 6-well (5×10^4/well) dishes, treated with the NC009 compounds for 8 h, and induced for TBP/Q_{79}-GFP expression for 6 days. Fluorogenic CellROX deep red reagent (5 μM, Molecular Probes) designed to measure ROS in live cells was added to the cells and incubated at 37 °C for 30 min. After washing with PBS, the cells (5×10^4) were then analyzed for green (GFP) and red (ROS) fluorescence on a flow cytometry system (Becton–Dickinson), with excitation/emission wavelengths at 488/507 nm (green) and 640/665 nm (red).

2.5. SH-SY5Y TBP/Q_{79}-GFP aggregation and neurite outgrowth assays

The established SH-SY5Y TBP/Q_{79}-GFP cells were seeded in 24-well (2×10^4/well) plate, with all *trans* retinoic acid (10 μM, Sigma-Aldrich) added at seeding time. At day 2, cells were treated with NC009 compounds (0.1 μM) for 8 h. Then doxycycline (5 μg/ml) was added and the cells were kept in the medium containing retinoic acid, doxycycline and compounds for 6 days. Then cells were stained with Hoechst 33342 (0.1 μg/ml) and the aggregation percentage was assessed by HCA as described. In addition, the morphologic differentiation of TBP/Q_{79}-GFP cells was assessed by using Metamorph microscopy automation and image analysis software (neurite outgrowth application module, Molecular Devices). To quantify neurite outgrowth, the relative outgrowth in untreated cells is set as 100%.

2.6. Western blot analysis and filter trap assay

Total proteins were prepared using buffer containing 50 mM Tris-HCl pH8.0, 150 mM NaCl, 1 mM EDTA pH8.0, 1 mM EGTA pH8.0, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100 and protease inhibitor cocktail (Sigma-Aldrich). After centrifugation for 5 min at 15,400 × g, soluble proteins (20 μg) were separated by SDS-polyacrylamide gel electrophoresis (10 − 12%) and blotted on to nitrocellulose membranes (Schleicher and Schuell). After blocking, the membrane was probed with TBP (1:500 dilution, Santa Cruz), heat shock protein family B (small) member 1 (HSPB1) (1:500 dilution, Santa Cruz), BH3 interacting domain death agonist (BID) (1:1000 dilution, Cell Signaling), cytochrome c, somatic (CYCS) (1:1000 dilution, BioVision), caspase 3 (CASP3) (1:1000 dilution, Cell Signaling), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000 dilution, MDBio) or β-Actin (1:5000 dilution, Millipore) at 4 °C overnight. Then horseradish peroxidase-conjugated donkey anti-goat, goat anti-mouse or goat anti-rabbit IgG antibody (1:5000 dilution, GeneTex) and chemiluminescent substrate (Millipore) were used to detect the immune complexes.

For filter trap assay, the prepared total proteins (20 μg) were filtered through a cellulose acetate membrane (0.2 μm pore size, Schleicher and Schuell), which was pre-equilibrated with 1 × PBS and 2% SDS, using a BRL dot-blot filtration unit. After washing and blocking, the membrane was stained with the TBP antibody and the immune complexes on the membrane were detected as described.

2.7. RNA interference

Lentiviruses with short hairpin RNA (shRNA) targeting HSPB1 (TRCN0000008753, target sequence CCGATGAGACTGCCGCCAAGT) and a negative control scrambled shRNA (TRC2.Void) were obtained from National RNAi Core Facility, Institute of Molecular Biology/