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FTY720 (Fingolimod) reverses α-synuclein-induced downregulation of brain-derived neurotrophic factor mRNA in OLN-93 oligodendroglial cells

Ismael Segura-Ulate, Barbara Yang, Javier Vargas-Medrano, Ruth G. Perez*

Texas Tech University Health Sciences Center El Paso, Department of Biomedical Sciences, Graduate School of Biomedical Sciences, Paul L Foster School of Medicine, 5001 El Paso Dr, El Paso, TX 79905, USA

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ABSTRACT

Multiple system atrophy (MSA) is a demyelinating neurodegenerative disorder characterized by accumulation of aggregated α-synuclein (aSyn) inside oligodendrocyte precursors, mature oligodendroglia, and neurons. MSA dysfunction is associated with loss of trophic factor production by glial and neuronal cells. Here, we report that recombinant wild type human aSyn uptake by OLN-93, an oligodendroglia cell-line, reduced brain-derived neurotrophic factor (BDNF) expression. Furthermore, OLN-93 cells stably transfected with human wild type or an MSA-associated mutant aSyn, A53E that produces neuronal and glial inclusions, reduced BDNF mRNA to nearly unmeasurable qPCR levels. Curiously, another MSA-associated aSyn mutant, G51D that also produces neuronal and glial inclusions, caused only a trend toward BDNF mRNA reduction in transfected OLN-93 cells. This suggests that oligodendrocyte-associated BDNF loss occurs in response to specific aSyn types. Treating OLN-93 cells with 160 nM FTY720 (Fingolimod, Gilenya®), a Food and Drug Administration (FDA) approved therapeutic for multiple sclerosis, counteracted BDNF downregulation in all aSyn OLN-93 cells. FTY720 also restored BDNF mRNA in OLN-93 cells treated with recombinant aSyn, as measured by qPCR or semiquantitatively on agarose gels. Immunoblots confirmed that FTY720 increased histone 3 acetylation in OLN-93, and chromatin immunoprecipitation assays showed increased acetylated histone 3 at BDNF promoter 1 after FTY720. Moreover, OLN-93 cells treated with valproic acid, a classic histone deacetylase inhibitor, confirmed that increasing acetylated histone 3 levels increases BDNF expression. Cumulatively, the data suggest that FTY720-associated histone deacetylase inhibition stimulates BDNF expression in oligodendroglial cells, raising the possibility that MSA patients may also benefit by treatment with FTY720.

1. Introduction

Synucleinopathies are aging-related neurodegenerative disorders characterized by the accumulation of aSyn aggregates inside neuronal and glial cells. These pathologies include Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) (Goedert and Spillantini, 1998; Spillantini, 1999; Spillantini and Goedert, 2000). In PD and DLB, aSyn accumulates inside neuronal cells, the very cells in that make it. However, in MSA, aSyn accumulates in the myelin-producing oligodendroglia (OLG) cells, which normally do not express aSyn (Reyes et al., 2014). Among the synucleinopathies, MSA stands out because it is a demyelinating neurodegenerative disorder that can progress from diagnosis to death within 5–10 years (Jellinger, 2014; Stefanova and Wenning, 2016). The hallmark lesions of MSA are the so-called Papp-Lantos bodies, also called glial cytoplasmic inclusions (GCI) that are enriched in aggregated aSyn in the cytosol of OLG cells (Jellinger, 2014; Miller et al., 2004; Wakabayashi et al., 1998). Similar lesions are also present in the oligodendrocyte precursor cells (OPC) that normally give rise to mature OLGs throughout life (May et al., 2014). GCIs in MSA are believed to cause

Abbreviations: AcH3, acetylated histone 3; aSyn, α-synuclein; BDNF, brain-derived neurotrophic factor; bp, base pair; DLB, Dementia with Lewy Bodies; FDA, Food and Drug Administration; GCI, glial cytoplasmic inclusions; HDAC, histone deacetylase; MSA, Multiple System Atrophy; PD, Parkinson's disease; rh-aSyn, recombinant human wild type α-synuclein; WT-aSyn, wild type human α-synuclein; OLG, oligodendroglia; OPC, oligodendrocyte precursor cells; RT, room temperature.

* Corresponding author. Texas Tech University Health Sciences Center El Paso, MSB1 4002, 5001 El Paso Dr, El Paso, TX 79905, USA.
E-mail address: ruth.g.perez@ttuhsc.edu (R.G. Perez).

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OLG cell death, which leads to neuronal demyelination and subsequent neurodegeneration (Jellinger, 2014; Stefanova and Wenning, 2016). As is the case for all demyelinating disorders, the loss of axonal myelination in MSA is accompanied by a loss of other supportive factors that OLGs normally provide to neurons, such as neurotrophic factors (Ettle et al., 2016; Ubhi et al., 2012).

In the past ten years, accumulating evidence suggests that aSyn is secreted by neurons and can spread cell-to-cell in a prion-like manner (Emmanouilidou et al., 2010; Goedert et al., 2010), though there are conflicting data regarding whether aSyn is normally expressed by OLGs (Djelloul et al., 2015; Miller et al., 2005). Abundant data suggest that MSA pathology occurs after aSyn uptake by OLG cells (Ettle et al., 2014; Kisos et al., 2012; May et al., 2014; Pukass and Richter-Landsberg, 2014), and that the source of aSyn inside OLGs is likely neuronally-derived (Reyes et al., 2014). Also, multiple lines of evidence show that aSyn accumulation in neurons and OLGs can induce abnormal gene expression, independently of aSyn-aggregation-related cell death (Goers et al., 2003; Kim et al., 2014; Ma et al., 2014; May et al., 2014; Siddiqui et al., 2012; Yuan et al., 2010; Zhou et al., 2013). The pattern of expression of BDNF in MSA brain suggests that atypical BDNF expression contributes to MSA pathology (Kawamoto et al., 1999). Furthermore, BDNF levels are reduced in MSA mice that express human aSyn under OLG- or OPC-specific promoters (Ubhi et al., 2010), and aSyn overexpressing OPC cells also downregulate BDNF expression (May et al., 2014). Thus, aSyn accumulation in OLGs is thought to cause a loss of trophic support to neurons.

Because many of the MSA models described above relied on aSyn overexpression in OPC or OLG cells, which may or may not be a natural mechanism for aSyn accumulation (Djelloul et al., 2015; Miller et al., 2005; Reyes et al., 2014), we sought to measure the impact of aSyn uptake versus aSyn overexpression on the expression of BDNF by OLG cells. Furthermore, we sought to establish whether deleterious effects of aSyn accumulation in OPC/OLG cells was specific, or might also be triggered by the synuclein family member, beta-synuclein (bSyn) (Jakes et al., 1994). In addition, we evaluated the pre-clinical efficacy of FTY720 (2-Amino-2-[2-(4-octyl-phenyl)-ethyl]-propane-1,3-diol hydrochloride) in OLG cells. This drug stands out as a first-line pharmacological intervention for MSA by its BDNF stimulatory effects in vitro and in vivo (Deogracias et al., 2012; Di Pardo et al., 2014; Efstathopoulos et al., 2015; Hait et al., 2015; Heinen et al., 2015; Miguez et al., 2015; Noda et al., 2013; Vargas-Medrano et al., 2014) and also because as an FDA approved drug, it could be fast-tracked as a neuroprotective MSA therapy as recently done for pediatric Rett syndrome patients (ClinicalTrials.gov identifier NCT02061137). With this in mind, we assessed the impact of FTY720 on BDNF expression in OLN-93 cells as it relates to MSA, while modulating aSyn accumulation by either uptake or overexpression. Finally, we evaluated the mechanism of action by which FTY720 influences BDNF expression in OLGs.

2. Materials and methods

2.1. Cell culture

OLN-93 cells were generously provided by Dr. Jeffrey D. Macklis (Harvard Stem Cell Institute, Boston, MA) and Christiane Richter-Landsberg (Carl von Ossietzky Universitat; Oldenburg, Germany). OLN-93 cells were grown in Dulbecco's Modified Eagle's Medium with high glucose (Sigma-Aldrich, St. Louis, MO, Cat.D5648-10X1L), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GE Healthcare Life Sciences HyClone Laboratories, Logan, Utah, Cat. SH300071.03). Non-transfected OLN-93 cells were grown in medium containing 50 U/mL penicillin and 50 μg/mL streptomycin. Transfected OLN-93 cell lines were grown in medium with added G418 sulfate (0.8 mg/mL) (Corning Life Sciences Inc., Corning, NY, Cat. 61-234-RG). Incubation was maintained at 37 °C with 10% CO_2.

2.2. Site-directed mutagenesis and transfection to establish stable aSyn OLN-93 cell lines

In order to generate plasmids to express G51D-aSyn and A53E-aSyn mutant forms, we used a pcDNA3.1 plasmid containing human wild type aSyn (WT-aSyn) that we have used before (Perez et al., 2002). Single amino acid substitutions were achieved using a QuickChange XL Site-Directed Mutagenesis kit (Agilent Technologies, Santa Clara, CA, Cat. 200516) following the manufacturer's protocol. The G51D-aSyn and A53E-aSyn mutations were then verified by automated DNA sequencing (University of Texas at El Paso Genomic Analysis Core Facility, Department of Biology).

OLN-93 cells were transfected in 6 wells plates using 1 μg of plasmid vector (pcDNA3.1) as a control or human aSyn plasmids (WT-aSyn, A53E-aSyn, or G51D-aSyn) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, Cat. 11668-019) and Opti-MEM (Thermo Fisher Scientific, Waltham, MA, Cat. 22600-134). A separate well was mock-transfected using no plasmid. Selection of stably transfected clonal cell lines was accomplished using G418 sulfate (1 mg/mL) with clones chosen after all mock transfected cells had died.

Monoclonal cell lines were obtained by the limiting dilution selection method. At 3–5 days after the initial seeding into 96 well plates, wells containing a single colony were identified and selected for transfer and expansion. Stably transfected OLN-93 clonal aSyn lines were then selected for comparison based on aSyn levels measured by immunoblotting and immunocytochemistry.

2.3. Drugs and aSyn or bSyn treatment

For all assays that included drugs or recombinant proteins treatments, cells were seeded and grown in antibiotic-free medium during the entire assay. FTY720 (AbMole BioScience, Kowloon, Hong Kong, Cat. M1712) was prepared in ethanol and cells were treated at 160 nM as in our earlier studies using MN9D dopaminergic cells (Vargas-Medrano et al., 2014). Similarly, valproic acid (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, Cat. sc-202378) was prepared in double distilled sterile water and cells were treated with 150 μM or 75 μM concentrations. Full length, non-tagged, recombinant human wild type aSyn and recombinant human wild type bSyn expressed in *E. coli* were purchased from rPeptide LLC (Bogart, GA, Cat. S-1001-2 and S-1003-2, respectively) and prepared as instructed by the manufacturer. Briefly, lyophilized recombinant aSyn and bSyn were reconstituted to a 1 mg/mL (~69 μM) solution using double distilled sterile water. Cells were treated with recombinant human aSyn or bSyn at 1 μM final concentrations.

2.4. Immunoblots

Protein concentrations in cell lysates were determined by the bicinchoninic acid assay (Smith et al., 1985). Total proteins (25–50 μg per lane) were separated by SDS-PAGE, transferred to nitrocellulose membranes, blocked with 5% non-fat dry milk, and then incubated with primary antibodies overnight at 4 °C. Primary antibodies for immunoblotting included anti-aSyn (Santa Cruz Biotechnology Inc., Cat. sc-7011-R) (1:200 dilution), anti-bSyn (Novus Biologicals, Littleton, CO, Cat. NB100-79903) (1:1000 dilution), anti-AcH3 (Lys9/Lys14) (Cell Signaling Technology, Inc., Danvers, MA, Cat. 9677) (1:500 dilution), anti-total histone H3 (Cell Signaling Technology Inc., Cat. 96C10) (1:500 dilution), anti-phosphorylated ERK1/2 (Tyr204) (Santa Cruz Biotechnology Inc., Cat. sc-7383) (1:200 dilution), anti-total ERK1/2 (Santa Cruz