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DOPAL is transmissible to and oligomerizes alpha-synuclein in human glial cells☆

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ABSTRACT

Introduction: Glial cytoplasmic inclusions (GCIs) containing alpha-synuclein (AS) are a neuropathologic hallmark of multiple system atrophy (MSA). Oligomerized AS is thought to be the pathogenic form of the protein. Glial cells normally express little AS, but they can take up AS from the extracellular fluid. 3,4-Dihydroxyphenylacetaldehyde (DOPAL), an obligate intermediate in the intra-neuronal metabolism of dopamine (DA), potently oligomerizes AS. In this study we tested whether DOPAL is taken up by human glial cells and augments intracellular oligomerization of AS.

Methods: DOPAL (exogenous or endogenous from co-incubation with PC12 cells) and AS (native or A53T mutant form) were added to the incubation medium of glial cells (glioblastoma or MO3.13 oligodendrocytes). Glial cellular contents of DOPAL and its intracellular metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were measured at up to 180 min of incubation. Glial cellular AS oligomers were quantified by Western blotting.

Results: Neither glioblastoma nor MO3.13 cells contained endogenous catecholamines or AS. Co-incubation of the cells with DA-producing PC12 cells produced time-related increases in DOPAL and DOPAC contents. Similarly, glial cellular DOPAL and DOPAC contents increased rapidly after addition of DOPAL to the medium. After addition of native or A53T-AS, intracellular AS also increased. Incubation of glial cells with both DOPAL and AS enhanced the intracellular oligomerization of native and A53T-AS.

Conclusions: DOPAL is transmissible to glial cells and enhances intracellular oligomerization of AS. An interaction of DOPAL with AS might help explain the formation of CGIs in MSA.

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1. Introduction

Multiple system atrophy (MSA) is a rare neurodegenerative disease characterized by putamen dopamine (DA) depletion (Goldstein et al., 2015) and by neuronal loss in several brain areas participating in the regulation of movement and autonomic outflows (Dickson et al., 1999). The pathological hallmark of MSA is glial cytoplasmic inclusions (GCIs), especially in oligodendrocytes (Papp et al., 1989). GCIs contain abundant alpha-synuclein (AS) (Tu et al., 1998), which has been implicated in MSA pathogenesis (Scholz et al., 2009). AS oligomers may be the pathogenic form of the protein (Winner et al., 2011).

Human oligodendrocytes express little if any AS (Miller et al., 2005), but brain neurons overexpressing human AS can transfer the protein to grafted oligodendrocytes (Reyes et al., 2014). It has been proposed that prion-like spread of AS in the brain causes MSA (Prusiner et al., 2015).

Concerning mechanisms of AS oligomerization in glial cells, addition of the oxidant hydrogen peroxide or of the membrane lipid docosahexaenoic acid to the incubation medium augments AS oligomerization in oligodendrocytes (Pukass and Richter-Landsberg, 2014); however, endogenous processes linking pathology in dopaminergic neurons with GCIs in glial cells remain poorly understood.

One such link might be 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL, an obligate intermediate in the intra-neuronal metabolism of DA, is produced continuously in the cytoplasm of dopaminergic neurons. DOPAL buildup is toxic (Panneton et al., 2010), via generation of reactive oxygen species (Anderson et al., 2011) and protein modifications (Rees et al., 2009). DA depletion in MSA putamen is associated with DOPAL buildup, from a combination of a vesicular storage defect and decreased aldehyde dehydrogenase (ALDH) activity (Goldstein et al., 2015). Importantly, in cell-free, cell culture, and in vivo systems DOPAL potently oligomerizes and aggregates AS (Burke et al., 2008).

For the concept to gain credence that DOPAL links abnormal dopaminergic neuronal function with AS oligomerization in glial cells, one must first demonstrate that (1) DOPAL is transmissible from DA-containing cells to glial cells, and that (2) within glial cells DOPAL taken up from the extracellular fluid oligomerizes AS (Fig. 1). The

☆ Main point: DOPAL is transmissible to glial cells, and inside glial cells DOPAL enhances the intracellular oligomerization of native and mutant A53T alpha-synuclein.

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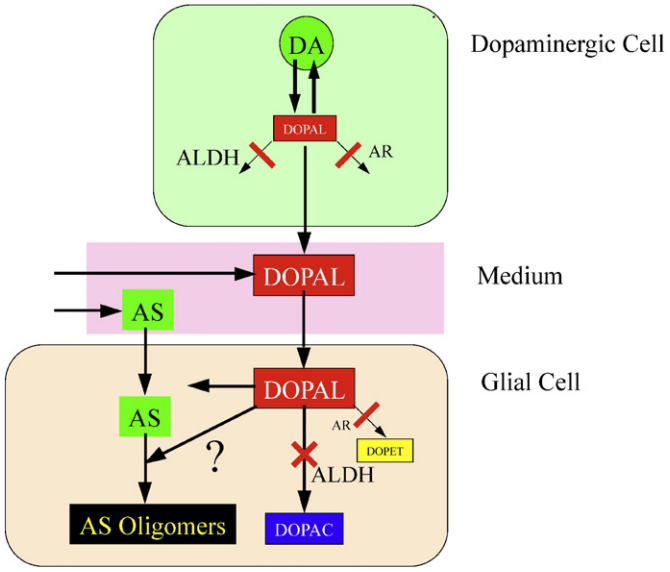

Fig. 1. Concept diagram depicting interactions between 3,4-dihydroxyphenylacetoaldehyde (DOPAL) and alpha-synuclein in glial cells. DOPAL, an obligate intermediate product of dopamine (DA) is toxic and highly reactive toward proteins. In the setting of inhibition of aldehyde dehydrogenase (ALDH) and aldehyde reductase (AR), endogenous DOPAL accumulates in PC12 cells and medium. The concept has two key elements: DOPAL can transfer from PC12 cells via the medium to glial cells; and co-incubation of DOPAL and alpha-synuclein augments alpha-synuclein oligomerization in glial cells.

present study was designed to provide such evidence. As a neutral catechol, DOPAL would be expected to diffuse freely across cell membranes.

Human cultured glioblastoma cells and MO3.13 oligodendrocytes were used. Glioblastoma cells are a cancerous cell line and relatively easy to grow, but one might question their applicability to oligodendrocytes, the main glial cell type containing AS inclusions in MSA. Human MO3.13 cells are an immortalized human-human hybrid cell line that upon differentiation expresses phenotypic characteristics of primary oligodendrocytes (Buntinx et al., 2003). In this study we tested both cell lines, to ascertain the reproducibility of results in different glial cellular models.

2. Materials and methods

2.1. Chemicals and reagents

Human recombinant AS was purchased from Calbiochem (La Jolla, CA, USA), mutant A53T-AS from rPeptide (Bogart, GA, USA). DA, L-DOPA, Dithiothreitol, benomyl, and Dulbecco's Modified Eagle's medium (DMEM) were from Sigma (St. Louis, MO, USA). DOPAL was from Santa Cruz Biotech (Dallas, TX, USA). All these reagents were dissolved in Type 1 water. Mouse anti-AS antibody and cell culture medium were from Invitrogen (Camarillo, CA, USA). Human glioblastoma (U118MG (ATTC^R HTB-15™), non-adherent PC12 cells, and F-12 K medium were from ATTC (Manassas, VA, USA) and human oligodendrocytes (MO3.13) from Cellutions Biosystems Ins (Burlington, Ontario, Canada). The aldehyde reductase inhibitor AL-1756 was a gift from Alcon Laboratories, Fort Worth, TX. Tolcapone was from Orion Pharma (Espoo, Finland). Benomyl, AL-1576, and tolcapone were dissolved in DMSO and stored at −20 °C. Co-incubation of PC12 cells with glial cells was done using ThinCert membranes from Greiner Bio-One North America Inc. (Monroe, NC, USA). For Western blotting NuPage LDS Sample Buffer (4X) (Invitrogen, ThermoFisher Scientific, Waltham, MA) containing sodium dodecyl sulfate (SDS) was used.

2.2. Experimental procedures

All the acute experiments were done using serum-free medium, to avoid DOPAL binding to the serum proteins. The experiments were done in triplicate.

2.2.1. Cell cultures

Human glioblastoma and MO3.13 cell were cultured in DMEM (with high glucose for MO3.13) containing 10% fetal calf serum. Glioblastoma (1×10^5 cells per well) or MO3.13 cells (5×10^4 cells per well) were prepared in 12-well plates. The PC12 cells used in this study were in suspension. The cells were cultured in F-12K medium containing 15% horse serum and 2.5% fetal calf serum. The PC12 cells were not differentiated. In our experience, with passaging these cells do not lose as much dopamine as readily as do adherent PC12 cells. Since the suspended cells have a high rate of dopamine synthesis without differentiation, we did not differentiate the cells. With differentiation, PC12 cells develop neurite-like extensions, making the cells more likely to clump and more difficult to count from well to well.

2.2.2. Tolcapone pre-incubation

At 24 h prior to all experiments, the medium was replaced by medium containing 10 μM tolcapone to block catechol-O-methyltransferase. At the beginning of the experiments, the medium was replaced again, this time with medium containing tolcapone with or without other inhibitors.

2.2.3. Assessment of enzymatic processes related to catecholamine synthesis or metabolism in glial cells

In cells, augmented DA production in the setting of DOPA incubation indicates L-aromatic-amino-acid decarboxylase activity; DOPAL or DOPAC production in the setting of DA incubation indicates monoamine oxidase (MAO) activity; and DOPAC production in the setting of DOPAL incubation indicates ALDH activity. Glioblastoma cells (1×10^5 cells) or MO3.13 cells (5×10^4 cells differentiated with β-phorbol 12-myristate 13 acetate for 3 days) were treated with 100 nmol DA, L-DOPA, or DOPAL for 180 min. Cells were collected in 400 μL of a 20:80 mixture of 40 mM H_2PO_4 with 200 mM acetic acid and disrupted by freezing and thawing. Glial cells were collected at different time points (0, 10, 20, 30, 60, 120, 180 min) and kept frozen at -80 °C until assayed for catechol contents.

2.2.4. DOPAL transmissibility from PC12 cells to glial cells

PC12 cells and glial cells were co-incubated separated by a membrane, so that the two cell lines shared the medium. PC12 cells (1.0×10^6 cells/well for glioblastoma cell experiments and 0.5×10^6 cells/well for MO3.13 experiments) were added to the insert within the well, which was humidified prior the experiment. Benomyl (1 μM) was added to the medium to decrease DOPAL metabolism by ALDH, and AL-1576 (1 μM) was added to decrease DOPAL metabolism by aldehyde reductase. These manipulations would be expected to maximize DOPAL accumulation in the medium. The glial cells were collected at 0, 10, 20, 30, 60, 120, 180 min of co-incubation with PC12 cells and kept frozen at -80 °C until assayed for cell catechol contents.

2.2.5. Glial cell uptake of AS

Native AS (3 μg/well) or mutant A53T-AS (3 μg/well) was added to the medium of glioblastoma cells (1×10^6 cell/well) and incubated for 300 min. The cells were collected, washed once with PBS, lysed with lysis buffer (20 mM Tris at pH 7.4, 25 mM KCl, 5 mM $MgCl_2$, 0.25 mM sucrose, 1% Triton X-100, and protease inhibitors), and analyzed for AS monomer and oligomers by Western blotting.

2.2.6. DOPAL-induced augmentation of AS oligomerization

Native AS (3 μg/well) or A53T-AS (3 μg/well) was added to the medium of glioblastoma cells (1×10^6 cell/well) or MO3.13 cells