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DJ-1 mutation decreases astroglial release of inflammatory mediators

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ABSTRACT

Mutations in *DJ-1*, reactive gliosis and concomitant inflammatory processes are implicated in the pathogenesis and progression of Parkinson's disease (PD). To study the physiological consequences of *DJ-1* mutation in the context of neuroinflammatory insult, primary cortical astrocytes were isolated from *DJ-1* knockout mice. Astrocytes were exposed to 1 μg/mL lipopolysaccharide (LPS) for 24 h following 2 h pre-exposure to inhibitors of MEK (U0126), JNK (JNK inhibitor II) or p38 (SB203580). Real-time PCR was used to assess the LPS-induced expression of pro-inflammatory mediators cyclooxygenase 2 (COX2), inducible nitric oxide synthetase (NOS2), and tumor necrosis factor α (TNFα). LPS-induced expression of COX2 decreased similarly in $DJ\text{-}1^{+/+}$ and $DJ\text{-}1^{-/-}$ astrocytes in response to inhibition of p38, but was unaffected by inhibition of MEK or JNK. No significant alterations in NOS2 expression were observed in any inhibitor-treated cells. The inhibitors did not affect expression of TNFα; however, $DJ\text{-}1^{-/-}$ astrocytes had consistently lower expression compared to $DJ\text{-}1^{+/+}$ counterparts. Secretion of TNFα and prostaglandin E2 (PGE2) into the culture medium was significantly decreased in $DJ\text{-}1^{-/-}$ astrocytes, and inhibition of p38 decreased this secretion in both genotypes. In conclusion, $DJ\text{-}1^{-/-}$ astrocytes may provide decreased neuroprotection to surrounding neurons due to alterations in pro-inflammatory mediator expression.

1. Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disease resulting from loss of dopaminergic neurons in the substantia nigra pars compacta. Approximately 5–10% of diagnosed PD cases have been associated with genetic mutations (Perrett et al., 2015). Specifically, mutations in 7 genes are robustly associated with autosomal dominant (SNCA, LRRK2, EIF4G1, VPS35) or recessive (parkin/PARK2, PINK1, DJ1/PARK7) PD (Bertolin et al., 2015; Mbefo et al., 2015; Puschmann 2013; Triplett et al., 2015).

DJ-1 is a recessive familial PD gene involved in anti oxidative function as well as mitochondrial maintenance (Cai et al., 2015; Saito et al., 2014). *DJ-1* is also known to regulate the activity of phosphatase and tension homolog (PTEN) which plays a critical role in neuronal cell death in response to various insults. DJ-1 protein is widely produced throughout mammalian tissues (Olzmann et al., 2004; Zhang et al., 2005; Larsen et al., 2007) and may function as an atypical peroxiredoxin-like peroxidase, capable of scavenging reactive oxygen species (Andres-Mateos et al., 2007) and mediate cellular protection through its antioxidant properties (Wilson, 2011). DJ-1 has been suggested to function as a redox-dependent chaperone, promoting proper folding of α-synuclein (Shendelman et al., 2004; Zhou et al., 2006). Further, DJ-1 has been shown to accumulate in brain tissue and is elevated in the plasma and cerebrospinal fluid of individuals with PD (Waragai et al., 2006; Waragai et al., 2007).

Given the myriad of key proteins that DJ-1 may modulate, including α-synuclein and Nrf2 (nuclear factor-like 2), DJ-1 could affect numerous physiological processes. Indeed, like PD, other neurological disorders have alterations in *DJ-1* expression and/or function including Alzheimer's disease (Choi et al., 2006), multiple system atrophy and Pick's disease, (Neumann et al., 2004), multiple sclerosis (Hirotani et al., 2008), cells expressing huntingtin (Goswami et al., 2006), and ischemia/reperfusion injury (Aleyasin et al., 2007; Yanagisawa et al., 2008), indicating DJ-1 may play a significant role in maintaining CNS homeostasis (Batelli et al., 2015). Although in humans *DJ-1* mutation induces PD, *DJ-1* knockout mice fail to consistently recapitulate the key clinical and neuropathological features of PD (Rousseaux et al., 2012; Yamaguchi and Shen, 2007) suggesting the existence of compensatory mechanisms that may protect mice from the neurodegeneration and the consequent motor symptoms. However, recent reports suggest consistent alterations in behavioral and striatal dopamine abnormalities in the *DJ-1* knockout mice (Hennis et al., 2013; Hennis et al., 2013). It is apparent that the *DJ*-1 mutant mouse model provides an excellent platform for investigating PD

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as a multi-factorial disease including the specific role of *DJ-1* in neural cells.

In utero exposure to bacterial lipopolysaccharide (LPS) has been shown to decrease the number of dopaminergic neurons as well as brain dopamine levels in offspring (Bakos et al., 2004; Carvey et al., 2003; Ling et al., 2002). Further, exposure to LPS as an adult can initiate neuronal cell loss within the substantia nigra in experimental models, and may be an environmental factor in the development of PD (Gayle et al., 2002; Herrera et al., 2000; Qin et al., 2007). LPS acts through toll-like receptors to increase the expression of numerous pro-inflammatory mediators in microglia and other cells, including interleukin1 (IL-1) (Sharif et al., 1993), interleukin 6 (IL-6) (Chung and Benveniste, 1990), mitogen-activated protein kinases (Tai et al., 2013), and TNFα (Liu et al., 2014). Recently, LPS-induced inflammation was demonstrated to induce mitochondrial dysfunction in the substantia nigra as well as the striatum, with increases in COX2 and NOS2 (Hunter et al., 2007). In animals, LPS also induces astrogliosis and neurodegeneration (Hoban et al., 2013), evidenced by increased glial-fibrillary acidic protein (Cai et al., 2003). Several studies have shown that astroglia and microglia differentially expression IL-1b and TNFα following stimulation by LPS, with microglia having higher expression levels of IL-1b but TNFα expression being comparable between the two cell types (Lu et al., 2014). Reactive astrocytes consistently have high expression of DJ-1 (Saito et al., 2014), suggesting that *DJ-1* has a significant role in gliosis or is elevated resultant to the gliosis (Bandopadhyay et al., 2004; Meulener et al., 2005). Interestingly, DJ-1 protein increases following exposure to LPS (Ejima et al., 2000; Mitsumoto and Nakagawa, 2001) which may indicate that DJ-1 might possibly play an important role in preventing or alleviating LPS-induced toxicity.

Increasing evidence indicates that development of PD represents a labyrinth of interactions occurring over time, with contributing factors including genetic predisposition and exposure to environmental toxins. Given that pro-inflammatory mediators promote neuronal viability and prolonged inflammation contributes to neurodegeneration, we tested the hypothesis that LPS-exposed astrocytes from DJ-1 mutant mice would exhibit alterations in expression of genes related to inflammation. *DJ-1's* role in modulating the inflammatory response may help to elucidate pathways involved in PD and other neurological diseases.

2. Materials and methods

2.1. Primary astrocyte isolation

DJ-1 mutant mice were generated, characterized, and genotyped as previously described (Ashley et al., 2009). Heterozygous mice were bred to maintain a colony containing all three genotypes. Primary mouse cortical astrocytes were isolated and maintained as previously described (Allen et al., 2000). This isolation method results in 98% pure astrocyte culture, as determined through immunofluorescent staining (Carbone et al., 2008). Cortices from day 0 to 1 old mouse pups were dissected and meninges removed. Tissue was digested with dispase (1.5 U/mL) in warm MEM with Earle's Salt and L-glutamine (Fisher) supplemented with an antibiotic cocktail (Invitrogen) containing penicillin (0.001 mg/mL), streptomycin (0.001 mg/mL), and neomycin (0.002 mg/mL). Cells were plated on tissue culture dishes (BD Biosciences) at 300,000 cells/mL in culture medium described above (without dispase) supplemented with 10% fetal bovine serum (Atlas). Culture medium was changed after 24 h to remove the non-adherent microglial cells. Astrocytes were cultured for 3–4 weeks to achieve approximately 80% confluence. Culture medium was changed 24 h prior to application of any treatment to minimize serum shock. All animal procedures were approved by the Colorado State University Institutional Animal Care and Use Committee.

2.2. Real-time PCR

To assess which protein(s) of the mitogen-activated protein kinase (MAPK) pathways are involved in COX2, NOS2, and TNFα increases following LPS exposure in astrocytes, we utilized a MEK inhibitor (MEKi, 10 μM U0126, Cell Signaling), a p38 inhibitor (p38i, 30 μM SB203580, Calbiochem), or an inhibitor of JNK (JNKi, 10 μM JNK Inhibitor II, Calbiochem). Each inhibitor was suspended in DMSO (Sigma) at concentrations designed to deliver equivalent volumes of DMSO. DMSO alone was used in our control group at the same volume as inhibitor-exposed groups (0.1%). The concentrations of inhibitors selected have been demonstrated to inhibit phosphorylation of the respective protein in mouse primary cortical astrocytes previously demonstrated within our laboratory (Moreno et al., 2008). Cells were preincubated with inhibitors and astrocytes were subsequently exposed to PBS or 1 μg/mL LPS (Sigma) for 24 h, culture medium was collected for analysis, and RNA was isolated *via* the RNeasy Kit (Qiagen) including on-column DNase treatment. cDNA was synthesized with iScript (BioRad) to assess expression of COX2, NOS2, and TNFα *via* real-time PCR. Relative expression of COX2 (5′ - GGA GTC TGG AAC ATT GTG AAC - 3′, 5′ - GTA GTA GGA GAG GTT GGA GAA G - 3′), *NOS2* (5′ - TCA CGC TTG GGT CTT GTT - 3′, 5′ - CAG GTC ACT TTG GTA GGA TTT G - 3′) or TNF α (5′ - GCA CCA CCA TCA AGG ACT C - 3′, 5′ - GAA AGG TCT GAA GGT AGG AAG G - 3′) was measured using a BioRad iCycler utilizing SYBR green incorporation (BioRad). Expression of COX2, NOS2, and TNFα in each sample was normalized to expression of β-actin (5′-GAC AGG ATG CAG AAG GAG ATT ACT G-3′, 5′-GCT GAT CCA CAT CTG CTG GAA-3′) *via* the delta–delta C_T method (Livak and Schmittgen, 2001) and is reported as relative expression. Each primer set was examined prior to use to assure consistent PCR efficiency, production of one specific amplicon and absence of primer-dimers. As a negative control, each RNA sample without reverse transcriptase was also analyzed to assure the lack of genomic DNA contamination. Each RNA sample was assayed in duplicate for each primer set assessed. The relative mRNA expression of COX2 and TNFα in each genotype was compared to *DJ-1*$^{+/+}$ astrocytes exposed to PBS and DMSO. In all PBS-treated samples, NOS2 expression was beneath the detection limit; therefore expression of NOS2 was analyzed relative to LPS-exposed *DJ-1*$^{+/+}$ levels.

2.3. Secreted TNFα, prostaglandin, and nitric oxide

Astrocytes were cultured as described, and medium was collected and stored at −80 °C for later analysis. TNFα levels in culture medium samples were determined *via* an enzyme-linked immunosorbent assay (ELISA, eBiosciences). Secreted levels of prostaglandin E2 were assessed with a monoclonal EIA kit with limit of detection of 39–25,000 pg/mL (Cayman Chemicals). Nitric oxide levels in culture medium were determined *via* nitrate/nitrite colorimetric assay (Cayman Chemicals).

2.4. Statistical analyses

All statistical analyses were performed with GraphPad Prism software. Means were analyzed using two way ANOVA, and when they significantly differed ($p < 0.05$), Tukey's post hoc test was employed. Four sets of astrocyte cell cultures were examined: *DJ-1*$^{+/+}$ treated with PBS, *DJ-1*$^{+/+}$ treated with LPS, *DJ-1*$^{-/-}$ treated with PBS, and *DJ-1*$^{-/-}$ treated with LPS. Each set contained four treatment groups: cultures were treated with MEKi, JNKi, or p38i, or with DMSO as the vehicle control for the inhibitors. mRNA levels were measured by real-time PCR in each group for genes of