Neuroscience 290 (2015) 454–471

INTERVENTION WITH 7,8-DIHYDROXYFLAVONE BLOCKS FURTHER STRIATAL TERMINAL LOSS AND RESTORES MOTOR DEFICITS IN A PROGRESSIVE MOUSE MODEL OF PARKINSON'S DISEASE ☆

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Abstract—Parkinson's disease (PD) is a progressive neurological disorder and current therapies help alleviate symptoms, but are not disease modifying. In the flavonoid class of compounds, 7,8-dihydroxyflavone (7,8-DHF) has been reported to elicit tyrosine kinase receptor B (TrkB) dimerization and autophosphorylation that further stimulates signaling cascades to promote cell survival/growth, differe ntiation, and plasticity. In this study we investigated if 7,8-DHF could prevent further loss of dopaminergic cells and terminals if introduced at the midpoint (i.e. intervention) of our progressive mouse model of PD. In our model, 1-methyl-4phenyl-1,2,3,6-tetrahyrdopyridine (MPTP) is administered with increased doses each week (8, 16, 24, 32-kg/mg) over a 4-week period. We found that despite 4 weeks of MPTP treatment, animals administered 7,8-DHF starting at the 2-week time period maintained 54% of the tyrosine hydroxylase (TH) levels within the dorsolateral (DL) striatum compared to the vehicle group, which was comparable to animals treated with MPTP for 2 weeks and was significantly greater compared to animals treated with MPTP for the full 4 weeks. Animals treated with MPTP and 7,8-DHF also demonstrated increased levels of, a sprouting-associated protein, superior cervical ganglion-10 (SCG10), phos phorylated TrkB (pTrkB), and phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2) within the DL striatum and substantia nigra (SN) compared to the 4-week MPTP-treated animals. In addition, motor deficits seen in the 2- and 4-week MPTP-treated animals were restored following administration of 7,8-DHF. We are reporting here for the first time that intervention with 7,8-DHF blocks further loss of dopaminergic terminals and restores motor deficits in our progressive MPTP mouse model. Our data suggest that 7,8-DHF has the potential to be a translational therapy in PD. Published by Elsevier Ltd. on behalf of IBRO.

Key words: 7,8-dihydroxyflavone, MPTP, mouse model, Parkinson's disease, motor behavior, collateral sprouting.

INTRODUCTION

Parkinson's disease (PD) is characterized by a progressive neurodegenerative loss of dopaminergic neurons in the nigrostriatal pathway. The decrease in dopamine (DA) within the basal ganglion circuit and the imbalance of DA and glutamate neurotransmitters in PD ultimately leads to progressive impairment of DA-stimulated motor behaviors (Meshul et al., 1999; Touchon et al., 2004; Holmer et al., 2005; Smith et al., 2011). It is hypothesized that many additive factors including genetic inheritance, pesticide exposure and aging contribute to the disease, though a definitive etiology of PD is unknown. Unfortunately there are no treatments available that slow down or prevent further loss of the dopaminergic neurons. However, deficits in certain biochemical markers, such as brain-derived neurotrophic factor (BDNF) or tyrosine kinase receptor B (TrkB), found in PD patients, could help in investigating potential alternative treatments.

Clinically it is well established that there is a significant decrease in the levels of BDNF in autopsy tissue taken from the midbrain of patients with PD (Mogi et al., 1999; Parain et al., 1999; Howells et al., 2000). Depletion of BDNF leads to motor deficits and dysfunction in striatal

☆ This work was supported by Merit Review #1BX 001643A to CKM from the United States (U.S.) Department of Veterans Affairs Biomedical Laboratory Research and Development. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

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Abbreviations: AKT, protein kinase B; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DA, dopamine; DAB, diaminobenzidine; 78-DHF, 7,8-dihydroxyflavone; DL, dorsolateral; ECF, enhanced chemifluorescence; EDTA, ethylenediaminetetraacetic acid; ERK1/2, extracellular signal-regulated kinase 1/2; Et/SA, 10% ethanol/saline; IHC, immunohistochemistry; MAPK, mitogen-activated protein kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; $P75^{NTR}$, p75 NT receptor; pAKT, phosphorylated protein kinase B; PBS, phosphate-buffered saline; PD, Parkinson's disease; pERK1/2, phosphorylated extracellular signal-regulated kinase 1/2; PI3K/AKT, phosphotidylinositol 3-kinase; pTrkB, phosphorylated tyrosine kinase receptor B; SA, normal saline; SCG10, superior cervical ganglion 10; SN, substantia nigra; SNpc, substantia nigra pars compacta; TBST, Tris-buffered saline tween 20; TH, tyrosine hydroxylase; TrkB, tyrosine kinase receptor B.

http://dx.doi.org/10.1016/j.neuroscience.2014.12.080
0306-4522/Published by Elsevier Ltd. on behalf of IBRO.

development (Li et al., 2012). Mechanistically BDNF is highly expressed in the central nervous system (CNS) (Lu et al., 2013) and is essential for dopaminergic and glutamatergic neurotransmission (Guillin et al., 2001; Carvalho et al., 2008). By binding to TrkB, BDNF causes the receptor to dimerize and autophosphorylate (Guillin et al., 2001; Carvalho et al., 2008). Activation of TrkB perpetuates a series of signaling cascades that promote cell growth/survival, synaptic plasticity, and neuronal differentiation by the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K/AKT) pathway, and the phospholipase C- γ1 pathway (Cobb, 1999; Grewal et al., 1999; Chao, 2003; Minichiello, 2009; Skaper, 2012). It has been reported that decreased levels of TrkB resulted in a significant loss of DA neurons in the substantia nigra pars compacta (SNpc) of aged mice and an increase in the sensitivity to further DA cell loss following administration to 1-methyl-4phenyl-1,2,3,6-tetrahyrdopyridine (MPTP) (Baydyuk et al., 2011). Interestingly, Benisty et al. (1998) reported that there was a decrease in the number of TrkB-labeled neurons in the SNpc in PD patients even though mRNA expression was unchanged. Whether deficits of BDNF and/or TrkB stimulation is an outcome or a cause of PD is unknown, however, finding a treatment to restore the levels of activated TrkB signaling might alleviate or slow down the progression of the disease.

Unfortunately, the pharmacokinetic profile of BDNF creates many challenges for drug delivery, likely due to a short half-life (Jang et al., 2010) and inability to cross the blood–brain barrier (BBB) (Pardridge et al., 1998; Ankeny et al., 2001; Nagahara and Tuszynski, 2011). Furthermore, similar to other gene therapy trials, using recombinant BDNF in clinical trials have been unsuccessful (Ochs et al., 2000; Thoenen and Sendtner, 2002; Bartus et al., 2014). An alternative approach to bypass the issues with BDNF therapy could be through systemic administration of the TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF). As a flavone derivative, 7,8-DHF has been shown to instigate the dimerization and autophosphorylation of TrkB just as well as BDNF (Jang et al., 2010). This subsequently activates both the MAPK and PI3K/AKT pathways robustly as demonstrated by an increase in levels of phosphorylated AKT (pAKT) and phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2) (Jang et al., 2010; Liu et al., 2010). 7,8-DHF specifically activates TrkB and protects neurons from apoptosis in a TrkB-dependent manner (Obianyo and Ye, 2013). Unlike BDNF, 7,8-DHF can cross the BBB and can be administered non-invasively. Further investigation of 7,8-DHF needs to be carried out in animal models of PD to gage it's therapeutic potential.

In our progressive MPTP mouse model of PD, animals are injected with MPTP for 4 weeks (5 days/week) with increasing doses each week (8, 16, 24, and 32-mg/kg). MPTP selectively lesions the dopaminergic neurons in the brain (i.e. basal ganglia) (Meredith et al., 2008; Tieu, 2011; Blandini and Armentero, 2012) and has been widely implemented in animal neurotoxin models of PD. The progressive nature of our model not only results in a more gradual neurodegeneration of the nigrostriatal pathway and motor/gait deficits (Goldberg et al., 2011a) but also better mimics the clinical progressive decline of nigrostriatal function. Another advantage to progressive doses of MPTP over a 4-week time period, is that treatments can be implemented at any time during the progression to test whether a particular treatment can slow or stop the gradual loss of nigrostriatal function.

In this study, we use the progressive MPTP neurotoxin model of PD and performed an intervention study where we started the administration of 7,8-DHF two weeks after the initial MPTP dosing regimen (i.e. intervention). MPTP and 7,8-DHF were each administered for a total of 4 weeks. We hypothesized that 7,8-DHF would slow down the loss of dopaminergic cells and terminals through a TrkB-dependent mechanism despite continued administration of MPTP.

EXPERIMENTAL PROCEDURES

Animals

60 male C57BL/6J mice (Jackson Labs, Bar Harbor, ME, USA; 8-weeks old at arrival) were housed 3–4/cage and maintained on a 12-h light/dark cycle throughout (lights on 0600). They had ad libitum access to food and water. Mice were randomized into six groups: eight mice in the vehicle group (VEH), 12 mice in the MPTP group (4WK_MPTP), eight mice in the vehicle/7,8-DHF group (VEH + DHF), 12 mice in the MPTP/7,8-DHF group (4WK_MPTP + DHF), eight mice in the 2-week vehicle group (2WK_VEH), and eight mice in the 2-week MPTP group (2WK_MPTP). An additional group of four mice were administered MPTP for 2 weeks and then were off any treatment for 4 weeks (2WK_MPTP_4WKoff). Handling and care of mice was consistent with federal guidelines of the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and protocols were approved by the Portland VA IACUC.

MPTP and 7,8-DHF administration

MPTP-treated animals received intraperitoneal injections 5 days/week for 4 weeks total. The dosing of MPTP increased with each week with 8-mg/kg for week 1, 16-mg/kg for week 2, 24-mg/kg for week 3, and 32-mg/kg for week 4. Normal saline (SA) (0.1 ml/0.1 kg) was used as the vehicle for MPTP.

7,8-DHF (TCI America, Portland, OR, USA) was dissolved in a 10% ethanol/saline (Et/SA) solution. The amount of ethanol used to dissolve 7,8-DHF was carried out according to the solubility as provided by the manufacture's datasheet. Et/SA was used as the vehicle for 7,8-DHF. 5-mg/kg of 7,8-DHF, as previously reported (Jang et al., 2010; Liu et al., 2010; Zhang et al., 2014), was administered intraperitoneally to animals within the treatment groups (i.e. 4WK_MPTP + DHF and VEH + DHF) beginning the third week of MPTP administration. 7,8-DHF was systemically administered for a total of 4 weeks (5 days/week) and all 7,8-DHF injections took place in the late afternoon (4–5 pm). When 7,8-DHF injections began, animals received two injections a day during the final two weeks of MPTP treatment; hence, MPTP