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COMPARATIVE STUDY OF THE NEUROTROPHIC EFFECTS ELICITED BY VEGF-B AND GDNF IN PRECLINICAL *IN VIVO* MODELS OF PARKINSON'S DISEASE

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Abstract—Vascular endothelial growth factor B (VEGF-B) has recently been shown to be a promising novel neuroprotective agent for several neurodegenerative conditions. In the current study we extended previous work on neuroprotective potential for Parkinson's disease (PD) by testing an expanded dose range of VEGF-B (1 and 10 μg) and directly comparing both neuroprotective and neurorestorative effects of VEGF-B in progressive unilateral 6-hydroxydopamine (6-OHDA) PD models to a single dose of glial cell line-derived neurotrophic factor (GDNF, 10 μg), that has been established by several groups as a standard in both preclinical PD models. In the amphetamine-induced rotational tests the treatment with 1 and 10 μg VEGF-B resulted in significantly improved motor function of 6-OHDA-lesioned rats compared to vehicle-treated 6-OHDA-lesioned rats in the neuroprotection paradigm. Both doses of VEGF-B caused an increase in tyrosine hydroxylase (TH)-positive cell and fiber count in the substantia nigra (SN) and striatum in the neuroprotective experiment. The effect size was comparable to the effects seen with GDNF. In the neurorestoration paradigm, VEGF-B injection had no significant effect in either the behavioral or the immunohistochemical analyses, whereas GDNF injection significantly improved the amphetamine-induced rotational behavior and reduced TH-positive neuronal cell loss in the SN. We also present a strong positive correlation ($p = 1.9e{-}50$) of the expression of *VEGF-B* with nuclear-encoded mitochondrial genes involved in fatty acid metabolism in rat midbrain, pointing to the mitochondria as a site of action of VEGF-B. *GDNF* showed a positive correlation with nuclear-encoded mitochondrial genes that was not nearly as strong ($p = 0.018$). VEGF-B counteracted rotenone-induced reduction of (a) fatty acid transport protein 1 and 4 levels and (b) both Akt protein and phosphorylation levels in SH-SY5Y cells. We further verified VEGF-B expression in the human SN pars compacta of healthy controls and PD patients, in neuronal cells that show co-expression with neuromelanin. These results have demonstrated that VEGF-B has potential as a neuroprotective agent for PD therapy and should be further investigated.

Key words: neuroprotective factors, rodent 6-OHDA model, human substantia nigra pars compacta, mitochondria, FATP1, FATP4.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease characterized by the cardinal movement symptoms bradykinesia, resting tremor, muscle rigidity, and postural abnormalities (Savitt et al., 2006; Olanow et al., 2009). Non motor symptoms of PD also include autonomic dysfunction, pain and sensory disorder, sleep impairment, and dementia (Olanow et al., 2009). The motor symptoms largely result from the degeneration of dopaminergic (DA) neurons projecting from the substantia nigra (SN) to the caudate putamen. PD symptoms appear after a loss of 70–80% of DA neurons has occurred and approximately 1–2% of the population over the age of 65 is afflicted with PD (Olanow et al., 2009).

The majority of PD patients are idiopathic with approximately 10% being familial (Farrer, 2006). Some environmental factors have been linked to an increased

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Abbreviations: AAV-2, adeno-associated virus; ACSVL, Acyl-coenzyme A synthetase very long; ANOVA, analysis of variance; ALS, amyotrophic lateral sclerosis; BSA, bovine serum albumin; CDNF, cerebral dopamine neurotrophic factor; *Chchd3*, coiled-coil-helix-coiled-coil-helix domain containing 3; *Chchd10*, coiled-coil-helix-coiled-coil-helix domain containing 10; *Coq9*, coenzyme Q9 homolog; *Cs*, citrate synthase; DA, dopaminergic; *Etfb*, electron-transfer-flavoprotein; FATP, fatty acid transport protein; *Fh1*, fumarate hydratase 1; GDNF, glial cell line-derived neurotrophic factor; *Hadhb*, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; *Idh3g*, isocitrate dehydrogenase [NAD] subunit gamma; i.p., intraperitoneal; MANF, mesencephalic astrocyte-derived neurotrophic factor; *Mcee*, methylmalonyl CoA epimerase; OD, optical density; 6-OHDA, 6-hydroxydopamine; *Ogdh*, oxoglutarate (alpha-ketoglutarate) dehydrogenase lipoamide; PBS, phosphate-buffered saline; PD, Parkinson's disease; PI, protease inhibitor; RT, room temperature; *Sdhb*, succinate dehydrogenase complex, subunit B, iron sulfur; *Sdhc*, succinate dehydrogenase complex, subunit C; *Sdhd*, succinate dehydrogenase complex, subunit D; SN, substantia nigra; SNpc, substantia nigra pars compacta; TBST, Tris-Buffered Saline + Tween 20; TH, tyrosine hydroxylase; VEGF-B, vascular endothelial growth factor B.

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risk of PD, for example rural well water use, pesticide use, and occupations such as mining or welding (Farrer, 2006). Although many genes have been identified, the exact mechanism in which the variation in PD-linked genes leads to neurodegeneration is not fully understood, however past research has pointed to mitochondrial dysfunction, oxidative damage, aberrant protein aggregation, and deficits in ubiquitin-mediated proteolysis (Olanow et al., 2009).

Current therapies are able to provide symptomatic relief but are unable to halt the progression of the disease (Olanow et al., 2009). A variety of neurotrophic factors, particularly those in the glial cell line-derived neurotrophic factor (GDNF)-family (GDNF and neurturin), have shown much promise in this regard in preclinical studies, demonstrating robust effects in rodent and primate models. For example, fibroblast growth factor (FGF; Timmer et al., 2007) has been shown to be neuroprotective. Others have been shown to be neuroprotective and neurorestorative in preclinical models of PD; for example GDNF (Hoffer et al., 1994; Kearns and Gash, 1995; Tomac et al., 1995; Gash et al., 1996; Kirik et al., 2004), neurturin (Horger et al., 1998; Rosenblad et al., 1999; Gasmi et al., 2007), as well as cerebral dopamine neurotrophic factor (CDNF; Lindholm et al., 2007; Voutilainen et al., 2011) and mesencephalic astrocyte-derived neurotrophic factor (MANF; Voutilainen et al., 2009).

Initial clinical trials of neurotrophic factors of the GDNF family, however, have not been successful likely because of a lack of information regarding the optimum dosing, delivery methods and choice of individual factors (Nutt et al., 2003; Lang et al., 2006). There is still reason to hope that trophic factor therapy may become a reality for patients with PD (Sherer et al., 2006) and experimental and clinical investigations to help clarify the potential role of GDNF and other neurotrophic factors to PD have been underway since. Several trials of viral gene therapy vectors in PD patients while not successful yet in slowing disease progression have shown the safety and tolerability of adeno-associated virus (AAV-2) gene delivery (Kaplitt et al., 2007; Marks et al., 2008, 2010; Christine et al., 2009; LeWitt et al., 2011), and Cere-120 (AAV2-neurturin) is currently being reevaluated in a second phase II clinical study with different injection sites and increased viral titer. It is therefore important to continue to evaluate novel growth factors in standard animal models concurrently with clinical trials now optimizing the delivery of traditional growth factors in the GDNF-family.

Vascular endothelial growth factor B (VEGF-B) is a substantially different growth factor with neuroprotective capabilities (Poesen et al., 2008; Dhondt et al., 2011), a member of the VEGF family (Rosenstein and Krum, 2004) rather than the GDNF-family. VEGF-A is the most studied VEGF family member but due to its angiogenic capabilities but is less suitable for use as a neurotrophic agent (Olsson et al., 2006). VEGF-B has very little angiogenic activity but it has the potential to inhibit apoptosis (Li et al., 2008) and increase stimulation in the proliferation of neuronal cultures *in vitro* (Sun et al., 2004). VEGF-B was up-regulated after exposing rat midbrain cultures to the pesticide rotenone (Falk et al., 2009a), a candidate environmental risk factor for PD (Tanner et al., 2011). Furthermore, exogenous supplementation of VEGF-B levels in this model system acted as a neuroprotective agent facilitating neuronal survival (Falk et al., 2009a). Based on those results VEGF-B was further evaluated as a putative neuroprotective agent *in vivo* (Falk et al., 2011) demonstrating that a 3-μg VEGF-B injection into the rat striatum after a mild progressive 6-hydroxydopamine (6-OHDA) was neuroprotective. The dose had been determined based on the lowest dose of other neurotrophic factors shown to be effective.

These findings have led us to further investigate effects of VEGF-B in both a neuroprotective and a neurorestorative preclinical PD model. In the present study we have tested an expanded dose range of VEGF-B (1 and 10 μg) and directly compared it to 10 μg GDNF, a dose that has been established by several groups and can be considered as a standard to use in the mild progressive 6-OHDA lesion rat PD model. Intrastriatal growth factor injection was conducted in rats either 6 h prior to 6-OHDA-lesioning to test neuroprotective effects, or 4 weeks after the lesion to test neurorestorative effects. After injection, behavioral tests and immunohistochemical analyses were conducted to measure any improvement in the diseased state. In order to further investigate the therapeutic potential for PD and understand the mechanism of action of VEGF-B, we also investigated VEGF-B expression in the human SN of PD patients, and effects of VEGF-B on fatty acid transport proteins (FATPs), sometimes also referred to as Acyl-coenzyme A synthetase very long (ACSVLs), and Akt signaling in rotenone-treated SH-SY5Y cells.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley (Charles River, Wilmington, MA, USA) rats were used in this experiment, weighing 250–280 g at the start. Rats were housed in groups of three on a 12-h light–dark cycle at room temperature (RT). Food pellets and water were available to them at all times. The experimental design was approved by the Institutional Animal Care and Use Committee at the University of Arizona and conformed to the guidelines of the National Institutes of Health.

Administration of 6-OHDA and the neurotrophic factors in the rat model

Neuroprotection paradigm. The unilateral 6-OHDA lesion was administered by injecting freshly made 20 μg 6-OHDA in one 4 μl deposit (Sigma, St. Louis, MO, USA; 5.0 μg/μl in 0.9% sterilized saline with 0.02% ascorbic acid) into the ventral lateral striatum at the following coordinates: A/P +0.8; L/P −2.5 and D/V −5.2 according to the atlas of Paxinos and Watson