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Regular Article

Increased brain bio-distribution and chemical stability and decreased immunogenicity of an engineered variant of GDNF

Rosamund C. Smith [a,*], Linda M. O'Bryan [a], Pamela J. Mitchell [a], Donmienne Leung [b], Mahmoud Ghanem [a,1], Jonathan M. Wilson [c], Jeff C. Hanson [d], Sandra Sossick [e], Jane Cooper [e], Lihua Huang [f], Kalpana M. Merchant [c,2], Jirong Lu [a], Michael J. O'Neill [e]

[a] Eli Lilly & Co., Biotechnology Discovery Research, Lilly Corporate Center, Indianapolis, IN 46285, USA
[b] Eli Lilly & Co., Lilly Biotechnology Center, 10300 Campus Point Dr, Suite 200, San Diego, CA 92121, USA
[c] Eli Lilly & Co., Tailored Therapeutics, Lilly Corporate Center, Indianapolis, IN 46285, USA
[d] Eli Lilly & Co., Information Technology, Lilly Corporate Center, Indianapolis, IN 46285, USA
[e] Eli Lilly & Co. Ltd, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK
[f] Eli Lilly & Co., Bioproduct Research and Development, Indianapolis, IN 46285, USA

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ABSTRACT

Several lines of evidence indicate that Glial cell line-derived neurotrophic factor (GDNF) is a trophic factor for dopaminergic neurons. Direct parenchymal administration of GDNF is robustly neuroprotective and neurorestorative in multiple neurotoxin-based animal models (rat and non-human primate (NHP)) of Parkinson's Disease (PD), suggesting its potential as a therapeutic agent. Although small, open-label clinical trials of intra-putamenal administration of bacteria-derived, full length, wild type GDNF (GDNFwt) were efficacious in improving standardized behavioral scores, a double-blinded, randomized controlled trial failed to do so. We hypothesize that the lack of clinical efficacy of GDNFwt in the larger randomized trial was due to poor bio-distribution in the putamen and/or poor chemical stability while in the delivery device for prolonged time periods at 37 °C. The development of neutralizing antibodies in some patients may also have been a contributing factor. GDNFv is an engineered form of GDNFwt, expressed and purified from mammalian cells, designed to overcome these limitations, including removal of the N-terminal heparin-binding domain to improve its diffusivity in brain parenchyma by reducing its binding to extracellular matrix (ECM), and key amino acid substitutions to improve chemical stability. Intra-striatal administration of a single injection of GDNFv in the rat produced significantly greater brain distribution than GDNFwt, consistent with reduced binding to ECM. Using liquid chromatography/mass spectrometry (LS/MS) methods GDNFv was shown to have improved chemical stability compared to GDNFwt when stored at 37 °C for 4 weeks. In addition, GDNFv resulted in lower predicted clinical immunogenicity compared to GDNFwt, as demonstrated by reduced CD4+ T cell proliferation and reduced IL-2-induced secretion in peripheral blood mononucleated cells collected from volunteers representing the world's major histocompatibility complex (MHC) haplotypes. GDNFv was demonstrated to be pharmacologically equivalent to GDNFwt in the key parameters in vitro of GFRα1 receptor binding, c-Ret phosphorylation, neurite outgrowth, and in vivo in its ability to increase dopamine turnover (DA). GDNFv protected dopamine nerve terminals and neurons in a 6-hydroxy-dopamine (6-OHDA) rat model. In summary, we empirically demonstrate the superior properties of GDNFv compared to GDNFwt through enhanced

Abbreviations: 6-OHDA, 6-Hydroxy-dopamine; BBB, Blood–brain-barrier; CEX, Cation Exchange chromatography; CHO, Chinese Hamster Ovary; DOPAC, Dihydroxyphenylacetic acid; Dopa, 3,4-dihydroxyphenylalanine; DA, Dopamine; ECM, Extracellular matrix; GDNF, Glial cell line-derived neurotrophic factor; GDNFv, GDNF variant; GDNFwt, *E. coli*-derived full-length native GDNF; GAP43, Growth associated protein 43; HVA, Homovanillic acid; HIC, Hydrophobic Interaction Chromatography; HLA, human leukocyte antigen; IHC, Immunohistochemistry; icv, Intra-cerebroventricular; kDa, kilodalton; L-Dopa, L-3,4-dihydroxyphenylalanine; LS/MS, liquid chromatography/mass spectrometry; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NGF, Nerve Growth Factor; NHP, Non-human primate; PD, Parkinson's Disease; TGF-β, Transforming Growth Factor beta; TH, Tyrosine hydroxylase; UPDRS, Unified Parkinson's Disease Rating Score.

* Corresponding author.
E-mail addresses: smith_ros@lilly.com (R.C. Smith), o'bryan_linda_maureen@lilly.com (L.M. O'Bryan), mitchell_pamela@lilly.com (P.J. Mitchell), leung_donmienne@lilly.com (D. Leung), mahmoud.ghanem@siemens.com (M. Ghanem), wilson_jonathan_matthew@lilly.com (J.M. Wilson), hanson_jeffrey_c@lilly.com (J.C. Hanson), sossick_sandra@lilly.com (S. Sossick), cooper_jane_x1@lilly.com (J. Cooper), huang_lihua@lilly.com (L. Huang), merchant.transthera@gmail.com (K.M. Merchant), lu_jirong@lilly.com (J. Lu), oneill_michael_j@lilly.com (M.J. O'Neill).
[1] Current address: Siemens Healthcare Diagnostics, 100 GBC Drive, Newark, DE 19702, USA.
[2] Current address: TransThera Consulting Co., 6702 S 850 E, Zionsville IN 46077, USA.

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bio-distribution and chemical stability concurrently with decreased predicted clinical immunogenicity while maintaining pharmacological and neurotrophic activity. These data indicate that GDNFv is an improved version of GDNF suitable for clinical assessment as a targeted regenerative therapy for PD.

Introduction

Several neuroprotective or neurotrophic molecules have been explored as potential treatments for PD. In particular, the neurotrophic ability of Glial cell line-derived neurotrophic factor (GDNF), a member of the Transforming Growth Factor-β (TGF-β) superfamily, as a potential treatment for PD has been extensively studied. Numerous studies report that the presence of GDNF facilitates the survival of dopamine neurons in culture (Lin et al., 1993) and protects mesencephalic primary neuronal cultures from 6-OHDA or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mediated cell death (Hou et al., 1996). These results have been confirmed in vivo using rodent models of PD (Fox et al., 2001). GDNF appears to act preferentially on dopaminergic neurons, and even a single injection of GDNF produces a profound increase in striatal dopamine content (Grondin et al., 2003; Hoffer et al., 1994). GDNF was effective at protecting and restoring dopaminergic neurons and terminals in both the 6-OHDA lesioned rat and MPTP-treated mouse models (Kearns and Gash, 1995; Kearns et al., 1997). Furthermore, post-lesion infusion of GDNF was able to restore tyrosine hydroxylase (TH) activity in the striatum and improve locomotion in 6-OHDA treated rats (Hoffer et al., 1994; Tomac et al., 1995). In addition to the pharmacological evidence in rodents, a genetic mouse with post-natal conditional knock-out of GDNF demonstrated that GDNF is indispensable for adult dopaminergic neuronal survival (Pascual et al., 2008). Thus, GDNF appears to have robust and reproducible neurotrophic activity in the dopamine-depleted nigrostriatal tract (Nakajima et al., 2001). There is some data to suggest that GDNF may have limited utility to rescue and protect neurons in an α-synuclein model of PD (Decressac et al, 2011; Lo Bianco et al., 2004). However, the restorative effect of GDNF has also been replicated in NHP models of PD (Gash et al., 1996; Grondin et al., 2002; Zhang et al., 1997). The anti-Parkinsonian action of GDNF in MPTP-treated monkeys was demonstrated with behavioral assessment using a primate-adapted version of the Unified Parkinson's Disease Rating Score (UPDRS). Post-mortem TH analysis showed repair of the dopaminergic nigrostriatal system in the GDNF-treated monkeys (Gash et al., 1996; Grondin et al., 2002).

A number of human PD trials with *E. coli*-derived, full-length, native GDNF (Liatermin; termed GDNFwt here) have been undertaken (Kordower et al., 1999; Nutt et al., 2001). The method of GDNFwt administration has been the subject of much debate since the protein does not easily cross the blood–brain-barrier (BBB) and systemic administration in humans causes deleterious side effects (Zurn et al., 2001). Intra-cerebroventricular (icv) administration of GDNFwt via an implanted catheter produced no improvements in Parkinsonism as measured by UPDRS and neuropathological evidence (Nutt et al., 2003). Gill et al. (2003) performed a more successful clinical trial in 5 PD patients by infusing GDNF directly into the putamen via a catheter pump device. After 12 months there was a 48% improvement in activities of daily living scores in the UPDRS and a significant reduction in L-Dopa induced dyskinesia (Gill et al., 2003). Striatal dopamine uptake was also increased in the putamen up to 18 months into the trial as assessed using functional imaging with F^{18}–Dopa. After two years on GDNF infusion, the patients had a 57% improvement in their off-medication motor scores and a 63% improvement in UPDRS (Patel et al., 2005). Imaging and functional performance measured in one of these patients up to 36 months after cessation of therapy demonstrated sustained benefit in both of these measures (Patel et al., 2013). Immunohistochemical (IHC) analysis of the brain of another one of these patients 3 months after cessation of therapy showed evidence for up-regulation of TH locally at the site of GDNFwt infusion in the posterior putamen and also increased expression of the sprout-associated protein, growth associated protein 43(GAP43), in the substantia nigra, providing evidence for GDNF-induced neuronal sprouting (Love et al. 2005). A second clinical study by Slevin and co-workers reported on the effects of 6-month unilateral intra-putamenal GDNFwt infusion in 10 patients with advanced PD (Slevin et al., 2005). Each patient was placed on a dose-escalation regimen of GDNF: 3, 10, and 30 μg/day at successive 8-week intervals, followed by a 1-month wash-out period. UPDRS total scores in the on and off states significantly improved 34 and 33%, respectively, at 24 weeks compared with baseline scores. In addition, UPDRS motor scores in both the on and off states significantly improved by 30% at 24 weeks compared with baseline scores. Thus in these small open labeled studies, GDNFwt, when delivered locally in the basal ganglia but not when administered distally via the ventricles, appeared to be sufficient to restore dopamine nigrostriatal activity and improve UPDRS scores. These small trials were followed up with a larger, randomized study (34 patients) in which GDNFwt (Liatermin) was infused into the putamen of PD patients for six months (Lang et al., 2006). While UPDRS scores improved in those patients given GDNF, it was not significantly different from those receiving placebo. In addition, dopaminergic activity (as measured by F^{18}–Dopa uptake) in the striatum of subjects given GDNF was only evident in the immediate tissue surrounding the infusion site.

The discordance between animal model data, open label trials and the randomized control trial has been a topic of much discussion. Gash et al. (2005) had previously demonstrated in Parkinsonian monkeys that the extent of GDNF coverage of the putamen predicted functional improvement. Subsequent analyses by Salvatore et al. (2006) indicated that the doses and catheter used in the Phase 2 GDNFwt trial may not have be optimal. Efforts to recapitulate the human brain distribution in monkey studies with GDNFwt administered via a catheter/pump device similar to that used by Lang et al. (2006) supported the hypothesis that lack of clinical efficacy was likely due to an insufficient coverage of the putamen, estimated to be 5-8% (Salvatore et al., 2006). Extrapolating from data generated in NHPs, it is estimated that wider coverage of the human putamen by GDNF, on the order of 30% or more, would be a key determinant of symptomatic efficacy.

Additionally, in the Phase II clinical study with GDNFwt , 18 out of 34 patients developed anti-drug antibodies and in 4 cases these antibodies were shown to be neutralizing (Tatarewicz et al., 2007). Neutralizing antibodies can not only limit efficacy of the drug but since GDNF is an endogenous protein with peripheral functions, it raises the possibility of blocking functions of peripheral endogenous GDNF. How GDNF reached the periphery and induced antibody formation in these patients was unclear but likely involved catheter movement, or aspects involving peripheral delivery (Tatarewicz et al., 2007). This observation raises concerns regarding the immunogenicity of GDNFwt, which potentially could impact both efficacy and safety of the drug, although no adverse consequences were reported in the trial (Tatarewicz et al., 2007). Finally, Piccinini et al. (2013) recently showed that GDNFwt expressed and purified from *E. coli* had poor stability, raising this as another limitation to GDNFwt as a therapeutic for PD, since the drug may need to reside in a pump/catheter system at body temperature for many weeks to months prior to infusion.

The goal of this study was to engineer a biologically active GDNF variant (GDNFv) with the following improved properties over GDNFwt