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CDNF Protein Therapy in Parkinson's Disease

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Abstract

Neurotrophic factors (NTF) are a subgroup of growth factors that promote survival and differentiation of neurons. Due to their neuroprotective and neurorestorative properties, their therapeutic potential has been tested in various neurodegenerative diseases. Bioavailability of NTFs in the target tissue remains a major challenge for NTF-based therapies. Various intracerebral delivery approaches, both protein and gene transfer-based, have been tested with varying outcomes. Three growth factors, glial cell-line derived neurotrophic factor (GDNF), neurturin (NRTN) and platelet-derived growth factor (PDGF-BB) have been tested in clinical trials in Parkinson's Disease (PD) during the past 20 years. A new protein can now be added to this list, as cerebral dopamine neurotrophic factor (CDNF) has recently entered clinical trials. Despite their misleading names, CDNF, together with its closest relative mesencephalic astrocyte-derived neurotrophic factor (MANF), form a novel family of unconventional NTF that are both structurally and mechanistically distinct from other growth factors. CDNF and MANF are localized mainly to the lumen of endoplasmic reticulum (ER) and their primary function appears to be modulation of the unfolded protein response (UPR) pathway. Prolonged ER stress, via the UPR signaling pathways, contributes to the pathogenesis in a number of chronic degenerative diseases, and is an important target for therapeutic modulation. Intraputamenally administered recombinant human CDNF has shown robust neurorestorative effects in a number of small and large animal models of PD, and had a good safety profile in preclinical toxicology studies. Intermittent monthly bilateral intraputamenal infusions of CDNF are currently being tested in a randomized placebo-controlled phase I–II clinical study in moderately advanced PD patients. Here, we review the history of growth factor-based clinical trials in PD, and discuss how CDNF differs from the previously tested growth factors.

Keywords

neurotrophic factors, neurorestoration, clinical trial, mechanism of action, endoplasmic reticulum stress, CDNF, MANF, GDNF

Introduction

Neurotrophic factors (NTF) are small proteins that support the growth, survival, and differentiation of developing and mature neurons, and protect them from injury and toxins. The first NTF to be discovered was nerve growth factor (NGF)[1,2]. Since the early research, begun already in the 1940s, a large number of NTFs has been discovered, and NTFs are today categorized into three main protein families: neurotrophins [NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4)], the glial cell line-derived neurotrophic factor (GDNF)-family of ligands [GDNF, neurturin (NRTN), artemin (ARTN) and persephin (PSPN)], and neuropoietic cytokines [e.g., ciliary neurotrophic factor (CNTF), interleukin-6 (IL-6) and cardiotrophin (CT-1)]. An additional distant member of the GDNF family ligands, a protein called Growth Differentiation Factor 15 (GDF15), which signals via Ret and binds to the GDNF family receptor alpha-like (GFRAL) co-receptor, was recently discovered[3,4].

Conventional NTF exert their effects on neurons by binding to receptors, which typically have ligand-binding domains on the cell surface and cytoplasmic tyrosine kinase domains, on the plasma membrane of the target cells[5–7]. Activation of NTF receptors triggers intracellular signaling

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cascades, in particular phosphatidylinositide-3-kinase (PI3 K)-Akt and Ras-mitogen-activated protein (MAP) kinase pathways, that promote neuronal survival and stimulate neurite outgrowth.

A Brief History of Growth Factor Therapy in Parkinson's Disease

There has long been interest in the therapeutic use of NTFs, particularly in neurodegenerative diseases[8–10]. The blood-brain barrier (BBB) creates a major challenge in clinical application of NTFs, as proteins do not pass the BBB, and, thus, need to be delivered intracranially. Parkinson's Disease (PD) is an attractive target for NTF-based therapy as the disease is characterized predominantly by the degeneration of a single cell type, the nigrostriatal dopamine neurons, in an anatomically defined area, representing a discrete therapeutic target[8]. Local delivery of therapeutic proteins with neuroprotective and neurorestorative properties to the nigrostriatal pathway, where the cell bodies of dopamine neurons located at the substantia nigra pars compacta (SNpc) project their axons to the dorsal striatum, is a feasible therapeutic approach. This is supported also by the widely accepted view that degeneration of nigrostriatal dopamine neurons starts with gradual loss of synapses, followed by axonal degeneration, functional impairment, and eventually culminating in cell death. The motor symptoms, as well as some of the non-motor symptoms, of PD are caused by striatal dopamine deficiency linked to the selective degeneration of nigrostriatal dopamine neurons and their fibers, which occurs in a progressive, slow manner over a long period of time. Thus, successful protection, regeneration, and functional recovery of the nigrostriatal pathway is expected to have disease-modifying effects slowing down the progression of the disease, which remains a major unmet need in treatment of PD.

After GDNF had shown robust neurorestorative effects on motor symptoms and nigrostriatal integrity in both 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-based animal models of PD[11–14], clinical studies were initiated with high expectations. In the first attempt to treat human PD patients with GDNF, monthly intracerebroventricular (i.c.v.) infusions of the protein were given to 50 PD patients for 8 months in a phase I–II clinical study. There was no improvement in the Unified Parkinson's Disease Rating Scale (UPDRS)[15]. Although in a previous non-human primate study, i.c.v. administration of GDNF resulted in functional recovery[11], it is plausible that, in the larger human brain, the i.c.v. administered GDNF protein never reached the nigrostriatal target neurons in sufficient quantities. In a small open-label phase I study with five PD patients, continuous intraputamenal infusion of GDNF resulted in progressive and sustained improvement of motor function and activities of daily living[16]. At 1 year, the off-medication motor sub-score of the UPDRS was improved by 39% ($P < 0.002$) and the activities of daily living sub-score by 61% ($P < 0.002$). The clinical improvement was associated with significant increases in nigrostriatal ^{18}F-dopa uptake in positron emission tomography (PET), suggesting neurorestorative effects at the cellular level. In another open-label phase I study in 10 patients, unilateral intraputamenal GDNF infusion also demonstrated >30% improvements in both on- and off-medication UPDRS total scores at 24 weeks ($P < 0.0001$ for both on and off, total UPDRS at 24 weeks vs baseline)[17]. The clinical effects were maintained for at least up to 9 months after the end of treatment[18]. Notably, unilateral administration of GDNF protein resulted in sustained bilateral effects. These encouraging open-label trials were followed by a randomized, placebo-controlled phase II study in 34 patients with continuous bilateral intraputamenal delivery of GDNF. At 6 months, there was no statistically significant difference in UPDRS scores between the placebo and GDNF groups[19]. Differently from the previous open-label studies that included intra-patient dose escalation schemes, all patients in this study received bilateral continuous intraputamenal infusion of GDNF at a single dose level throughout the study. In addition, there were some differences between the types of in-dwelling brain catheters used in the three studies. Importantly, the drug delivery system and infusion protocol used in the phase II study was later tested in Rhesus macaques, and the results showed point-source concentration and steep concentration gradient of GDNF within putamen, suggesting that the limited volume of distribution of GDNF may have contributed to the lack of efficacy in this trial[20]. The authors of this paper calculated based on their non-human primate data that the bioavailability of GDNF may have been limited to only 2–9% of the putamen in human subjects in the phase II GDNF study. In addition, three of the patients who had received GDNF in the phase II study were reported to have developed neutralizing antibodies to GDNF indicating potential systemic leakage of the drug delivery system. Formation of auto-antibodies is a particular concern, as this would not only limit the efficacy of the protein therapeutic, but could also interfere with the function of the patient's endogenous GDNF. The disappointing results from the phase II study, together with findings of Purkinje cell loss in cerebellum in a few GDNF-treated monkeys[21], resulted in discontinuation of the GDNF program by Amgen. Importantly, case reports of patients who had received GDNF in the open-label studies showed that clinical improvement remained for several years after the studies had ended[22,23].

A new randomized, placebo-controlled, double-blind phase II clinical study in 41 PD patients was conducted recently using intermittent convection-enhanced intraputamenal delivery of GDNF protein (ClinicalTrials.gov identifier NCT03652363). In the main study, the patients received monthly infusions of GDNF, at a single dose of level of 120 μg per putamen, or placebo for 9 months, followed by a 9-month open-label study where all patients received GDNF at the same dose level. The improved drug delivery device and method is expected to provide better coverage of the putamen with the active drug infusate, compared with previous GDNF clinical studies. Importantly, this is the first clinical trial where GDNF was delivered once a month to the putamen, whereas all previous trials used continuous infusion of the trophic factor. In the intention-to-treat population at 9 months, the motor UPDRS scores in off-state did not significantly differ between GDNF and placebo groups (mean improvements $17.3 \pm 17.6\%$ vs 11.8 ± 15.8 from baseline, $P = 0.41$, respectively). A post hoc analysis found nine (43%) patients in the GDNF group but no patients in the placebo group with a large clinically significant motor improvement ($\geq$10 points) in the off state ($P = 0.0008$). Importantly, PET imaging showed a significant increase in ^{18}F-dopa uptake throughout the putamen only in the GDNF group, ranging from 25% (left anterior putamen; $P = 0.0009$) to 100% (both posterior putamina; $P < 0.0001$)[24]. After the 9-month open-label study where all patients received 120 µg of GDNF per putamen every 4 weeks, UPDRS in off-state decreased by $26.7 \pm 20.7\%$ in patients on GDNF for 18 months (GDNF/GDNF; $n = 21$) and $27.6 \pm 23.6\%$ in patients on placebo for 9 months followed by GDNF for 9 months (placebo/GDNF, $n = 20$; $P = 0.96$)[25]. No treatment-emergent safety concerns were identified. These results suggest that intermittent intraputamenal convection-enhanced delivery of GDNF produced a putamen-wide tissue engagement effect, overcoming prior drug delivery limitations. However, in comparison to effective dose levels of GDNF determined in non-human primate studies, the dose level of GDNF used in this study was significantly lower and suggest that stronger effects could be seen with optimal dosing level. Moreover, in this study PD patients with motor symptom duration for $\geq$5 years, and with moderate disease severity in the OFF state (Hoehn and Yahr stage 2–3 and UPDRS motor score (part III) between 25 and 45) and motor fluctuations were included. GDNF studies in animal models of PD support the view that GDNF treatment maybe more effective in earlier stages of disease when the caudate putamen has more GDNF-responsive dopaminergic nerve fibers.

An alternative delivery strategy was chosen for NRTN clinical studies. NRTN is a member of the GDNF family of ligands (GFLs) shown to provide robust trophic support for the nigrostriatal dopamine neurons[26,27]. NRTN signals through the Ret receptor, as does GDNF, but uses a different co-receptor (GFRα2 instead of GFRα1; although NRTN can mediate its signals to Ret also via GFRα1)[7]. Thus, similar neurorestorative effects are expected from both GDNF and NRTN in the injured nigrostriatal pathway. Continuous expression in the putamen by injection of adeno-associated virus serotype-2 (AAV2)-neurturin (CERE-120) was used to overcome the drug delivery issues in previous clinical trials with NTFs. Although, CERE-120 was intended to provide a lifetime of NTF support following a single administration, which may offer some advantages, the risk of this approach is that in case adverse effects would arise, turning off the expression of NRTN expression would not be possible. The CERE-120 construct also included some additional re-engineering for improving secretion of the mature protein. Most growth factors are first synthesized as immature proteins containing pre-pro sequences that guide maturation and secretion of the protein, and which are cleaved off from the mature growth factor. Since the AAV2 construct with native NRTN pre-pro sequence resulted in very poor secretion of NRTN, in the clinically used AAV2 construct it was replaced with the pre-pro sequence of NGF[28].

An open-label phase I study showed good safety and tolerability for CERE-120 in human PD patients[29]. In a randomized, sham surgery-controlled phase 2 study, CERE-120 was not superior to sham surgery based on the primary endpoint UPDRS motor score at 12 months[30]. Interestingly though, a subset of patients who had a longer blinded follow-up (for up to 18 months) showed a small but significant benefit in favor of CERE-120. Also, it should be noted that separation of the placebo group from the group receiving CERE-120 was not evident until 6–9 months after dosing suggesting a long-lasting placebo effect[28]. Considering the therapeutic outcome timeline, it is important to note that after intraparenchymal injection of AAV2 virus vector particles, protein expression is expected to start by 1 week and reach steady-state levels by 4 weeks post-injection[31]. Importantly, follow-up analysis revealed significant difference in response of early ($\leq$5 years after diagnosis) and late-stage ($\geq$10 years after diagnosis) PD patients to CERE-120. In early-stage patients treated with CERE-120, a clear trend of improvement in motor scores was seen in comparison to the placebo group. At the same time, no improvement was observed in late-stage patients who had received CERE-120[32]. A post-hoc comparison of CERE-120-treated patients stratified in two groups (PD diagnosis $\leq$5 years or $\geq$10 years before treatment start) showed a significant difference in treatment response in terms of UPDRS scores, in favor of the $\leq$5 years since diagnosis group ($P = 0.005$)[33]. The fact that, in patients with disease duration of $\geq$10 years before treatment, the vast majority of nigral dopamine neurons have already died[34] can explain this difference. Since GFLs, and NTFs in general, can rescue living neurons, the reported lack of CERE-120 efficacy in clinical trials in late-stage PD patients was not surprising.

These data also suggest that there may be a delayed benefit with gene transfer-based delivery of NRTN in PD patients, and, for the first time, showed a disease-modifying effect in human patients treated with a NTF. Due to concerns of deficiency in retrograde axonal transport in advanced PD patients, CERE-120 has been bilaterally administered to both putamen and SN in a small safety study, which supported feasibility of this approach[35]. The gene transfer approach is also currently tested for GDNF in an on-going open-label, single-center phase I dose escalation study investigating the safety and tolerability of AAV2-GDNF in advanced PD patients (ClinicalTrials.gov identifier NCT01621581).

PDGF-BB is a homodimer of the platelet-derived growth factor isoform B that has been shown to have restorative effects in the dopaminergic system *in vivo*[36,37]. While the exact mechanism of the neurorestorative effect of PDGF-BB remains to be defined, it has been hypothesized that stimulation of periventricular cell proliferation[37] and pericyte secretion of neuroregenerative molecules[38] would indirectly mediate these effects. A randomized, placebo-controlled phase I–IIa clinical study with 2-week continuous i.c.v. infusion of PDGF-BB was conducted in patients with moderate PD, with a 3-month follow-up period[39]; i.c.v. PDGF-BB was safe and well tolerated. While clinical rating scales showed no change between treatment groups, patients receiving the highest dose of PDGF-BB showed a significant increase in dopamine transporter (DAT) ligand binding in PET scans, compared with placebo patients who showed signal decline indicating on-going neurodegeneration[39]. At the end of the 3-month follow-up, there was an improvement in UPDRS part III motor scores in all cohorts, including the placebo group. In late 2015, it was announced that clinical development of intracerebral PDGF-BB in PD has been discontinued (Newron S.p.A., press release October 28, 2015). Growth factor-based clinical trials in PD are summarized in Table 1.

Lessons Learned: from Manufacturing to Clinical Study Design

There is a large number of potential explanations that may have contributed to outcome variability between preclinical and clinical studies using growth factors in treatment of PD, and several comprehensive reviews have been recently published discussing lessons learned from previous NTF clinical trials[32,40,41]. Here, we first briefly discuss issues related to the therapeutic approach, molecular properties of the investigational drug, manufacturing, preclinical, and clinical study design considerations, and, in the next section, focus more specifically on challenges of the intracranial drug delivery.

From the mechanistic perspective, only two mechanisms of action have been tested so far in the previous NTF clinical trials in PD patients. While the exact mechanism behind PDGF-BB action remains poorly understood, both GDNF and NRTN promote survival of dopamine neurons and regeneration of axons via the same pathway involving Ret-dependent activation of PI3K-Akt and Ras-MAP kinase signaling cascades. New molecular entities and mechanisms of action should be tested in the future, even in the context of growth factors. Particularly, as α-synuclein and neuroinflammation are important players in PD pathogenesis, they deserve more attention. In this regard, preclinical testing in moderate-to-severe lesion models in aged animals (both rodents and non-human primates) but also in α-synuclein-based models and in different lines of human dopamine neurons generated from PD patient-derived induced pluripotent stem (iPS) cells should be performed to build comprehensive understanding of the therapeutic potential of the investigational drug. Ideally, patient selection criteria in clinical studies should reflect those mechanisms that were effectively targeted in preclinical studies.

Factors likely to affect the efficacy of intracerebral growth factor therapies include the biological activity and formulation of the therapeutic, proper construct design of viral vectors (e.g. pre-pro sequences and promoters determining expression and secretion efficiency) and compatibility with the infusion device components, as well as disease-stage dependent efficiency of retrograde transport from putamen to SN. One critically important issue is the source and quality of the recombinant protein. The recombinant human GDNF used in the clinical trials was produced in *Escherichia coli*, which has later turned out to be less potent when compared with GDNF produced in mammalian cells[42]. In mammalian cells, GDNF and NRTN are synthesized as prepro-proteins. In the secretory pathway, GDNF and NRTN fold along with disulfide bridge formation, GDNF is modified by N-linked glycosylation at Asn49, and both proteins undergo proteolytic processing and homodimerization. GDNF and NRTN are cysteine knot proteins with three intramolecular S–S bridges and one S–S bridge holding together the dimers (Fig 1). After production in *E. coli*, unglycosylated GDNF is renatured *in vitro*. Experimental evidence demonstrates that both glycosylated and unglycosylated GDNF from mammalian cells are more stable than the *E. coli*-produced chemically renatured GDNF, and that the biological activity of the *E. coli*-produced GDNF batches varies[42].

Proper folding, post-translational modifications, batch-to-batch variation and stability of the recombinant protein need careful attention when manufacturing biologicals for clinical use. The critical quality attributes of the investigational protein product need to be carefully monitored, preferably using orthogonal methods, to ensure consistency in the manufacturing process and potency of the investigational protein. Analytical chemistry methods used for product characterization should take into account subtle changes that may occur to the product between manufacturing batches and during storage, such as charge, hydrophobicity, glycosylation, disulfide bridging, terminal modifications, aggregation, and levels of host cell contaminants (DNA, protein) and other impurities. Similarly, a panel of binding and potency assays should provide a comprehensive view on the quality and stability of the investigational protein.

Compatibility of the investigational protein product with the drug delivery device components should also be assessed to verify that biologically active protein is delivered to the target tissue. Importantly, gene therapy products may also suffer from problems associated with the product properties. For example, due to poor expression and secretion of the protein, the NRTN cDNA in CERE-120 was re-engineered to contain a pre-pro sequence from human NGF[28]. Notably, furin expression in the target cells in the putamen is very low, likely resulting in poor processing of the pre-pro sequence[47]. It is possible that an incompletely processed recombinant NRTN protein containing the NGF pro

Table 1. Clinical Trials with Growth Factors in Parkinson's Disease.

Growth factor	Delivery	Dosing	Phase	Patients	Stage	Key findings	References
GDNF	Lateral ventricle	rhGDNF, monthly bolus for 8 months	I–II, placebo controlled	50	H&Y 3–4 (off)	No improvement in UPDRS (off) as drug did not reach the target, various AEs (sensory symptoms, weight loss etc.).	Nutt et al[15]
	Putamen	rhGDNF, continuous infusion	I, open-label	5	Advanced (>6 years from diagnosis)	Safe and well-tolerated. Improvement in motor symptoms (UPDRS, off), [^{18}F]dopa uptake increased near the catheter tip (PET).	Gill et al[16]
	Putamen (unilateral)	rhGDNF, continuous infusion	I, open-label	10	H&Y 3-4 (off)	Improvement in motor symptoms (UPDRS, off), effects maintained 9 months after end-of-treatment.	Slevin et al[17,18]
	Putamen	rhGDNF, continuous infusion	II, placebo controlled	34	Advanced (>5 years from diagnosis)	No improvement in UPDRS (off), some increase in [^{18}F]dopa uptake (PET), development of anti-drug antibodies.	Lang et al[19]
	Putamen	rhGDNF, CED bolus for 9+9 months*	II, placebo controlled	41	H&Y ≤3 (off; >5 years from diagnosis)	No improvement in UPDRS (off), significant increase in [^{18}F]dopa uptake (PET).	Whone, Luz et al[24]; Whone, Boca et al[25] NCT03652363
	Putamen	AAV2-GDNF	I, open-label	25	H&Y 3-4 (off; >5 years from diagnosis	Study on-going. No results available yet.	NCT01621581
Neurturin	Putamen	AAV2-NRTN	I, open-label	12	H&Y 3-4 (off; >6 years from diagnosis	Safe and well-tolerated. Improvement in motor symptoms (UPDRS, off), no change in [^{18}F]dopa uptake (PET).	Marks et al[29]; NCT00252850
	Putamen	AAV2-NRTN	II, sham surgery controlled	58	Advanced(>5 years from diagnosis	AAV2-NRTN was not superior over sham surgery (UPDRS at 12 months).	Marks et al[30]; NCT00400634
	Putamen + SN	AAV2-NRTN	I, open-label	6	H&Y 2-3 (off; >4 years from diagnosis)	Safe and well-tolerated.	Bartus et al[35]; NCT00985517
PDGF-BB	Lateral ventricle	rhPDGF-BB, continuous infusion	I–II, placebo controlled	12	H&Y 2.5-3 (off; >5 years from diagnosis)	Well-tolerated. No change in clinical rating scales. [^{11}C]PE2I DAT binding increased in right putamen (PET).	Paul et al[39]; NCT02408562
CDNF	Putamen	rhCDNF, CED bolus for 6+6 months*	I–II, placebo controlled	18	H&Y 2.5-3 (off; >5 years from diagnosis)	Study on-going. Topline results expected in early 2020.	NCT03295786

sequence may induce apoptosis, if secreted[48]. Thus, verifying that biologically active protein is delivered to the target cells requires special attention regardless of the delivery approach. This aspect may have been insufficiently addressed in many of the previous clinical trials with NTFs. One alternative strategy that would allow avoiding the challenges associated with intracranial delivery of biological drugs is to develop small-molecule mimetics of NTFs that could be administered peripherally[49].

Properly powered, randomized, double-blinded, placebo-controlled studies should be preferred despite their higher cost and complexity. Although widely used as a primary

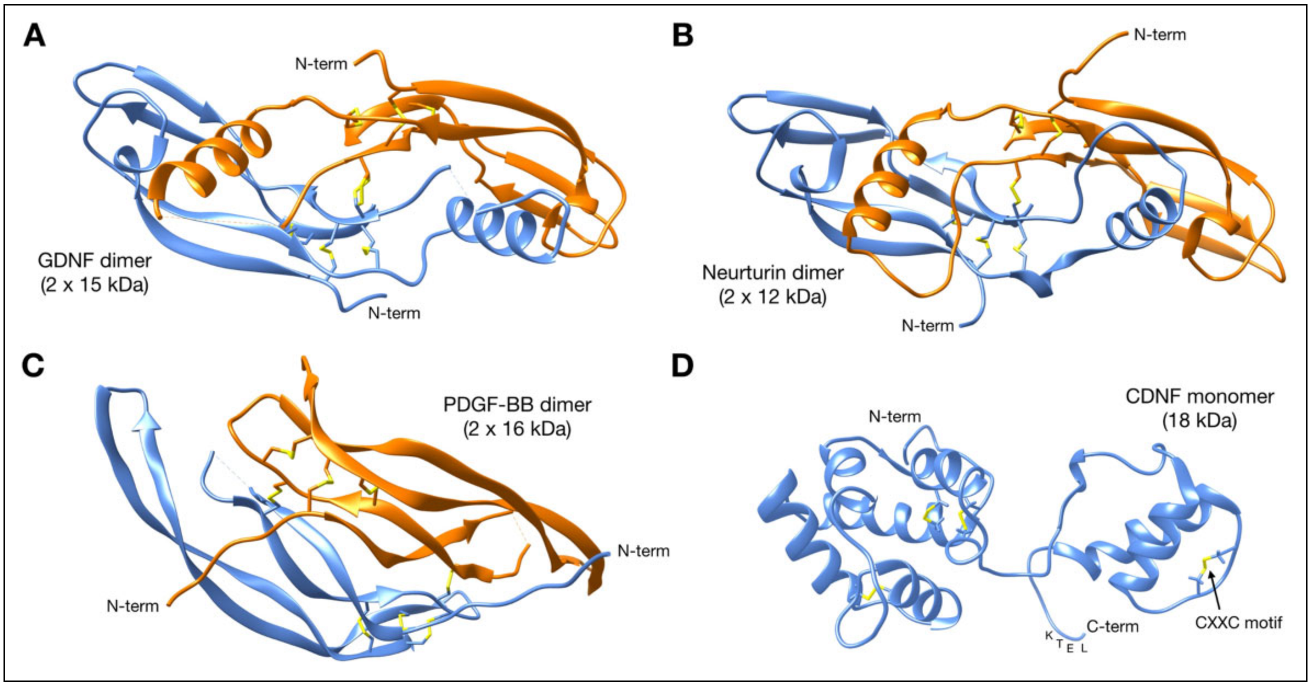

Fig 1. Structures of growth factors tested in human Parkinson's Disease patients. GDNF, NRTN, and PDGF-BB have typical growth factor-like dimeric structures with predominantly β-sheet secondary structures. CDNF has a distinct two domain, monomeric structure composed of α-helices only, and contains a CXXC motif and a C-terminal ER-retention sequence (KTEL). The structures displayed here were retrieved from PDB and have the following PDB IDs: 1AGQ (GDNF)[43], 5NMZ (NRTN)[44], 1PDG (PDGF-BB)[45], and 4BIT (CDNF)[46].

outcome measure in PD clinical studies, UPDRS is not well-suited for small studies, which is often the case with intracranially delivered therapeutics. More objective and practical clinical tools, also capable of assessing daily fluctuation of disease symptoms and non-motor symptoms, for assessing clinical improvement would be welcome. For example, digital wearable medical devices, such as the Parkinson's KinetiGraph™ system, allow continuous objective recording of movement symptoms and provide a valuable additional tool for assessing clinical outcome measures in PD studies[50,51]. Finally, inclusion of PET imaging for assessment of nigrostriatal pathway integrity is critically important in clinical studies with neurorestorative therapies. Development and validation of novel imaging biomarkers for reliable assessment of α-synuclein pathology and neuroinflammation will hopefully support clinical development of disease-modifying therapies in the future.

Lessons Learned: Challenges of Intracranial Drug Delivery in Clinical Trials

The clinical studies conducted so far with intracranially administered growth factors clearly indicate that the drug delivery method and the neuropharmacokinetic profile of the therapeutic compound are critical determinants of neurorestorative effects. Some potential explanations behind different results from the open-label and randomized clinical studies using intraputamenal GDNF infusion have been published pointing to technical differences in the drug delivery device and infusion protocol that may have resulted in poor biodistribution of GDNF in the target tissue[20]. Another factor limiting biodistribution of intraparenchymally infused GDNF and NRTN is associated with their molecular properties. Both GDNF and NRTN bind strongly to heparan sulphate-type glycosaminoglycans that are abundant on cell surfaces and extracellular matrix, which strongly limits their diffusion in tissue[49,52–54]. Coinfusion of heparin with GDNF and NRTN significantly increases their volume of distribution in animal models[55], but this approach is unlikely to be a clinically useful solution for improving biodistribution of intraputamenal GDNF and NRTN. NRTN is a poorly secreted and a poorly soluble protein, with a particularly high affinity to heparan sulphates. NRTN variants that were engineered to have reduced heparin-binding activity showed increased solubility and stability, as well as broader diffusion in the brain, which correlated with enhanced regenerative effects in the 6-OHDA rat model of PD[54]. Similarly, a novel GDNF variant with reduced heparin-binding capacity showed improved brain diffusion[52,56]. One caveat of this approach is that deletion of heparin-binding regions from GDNF may have undesired consequences, such as altered SorLA-mediated trafficking of GDNF-GFRα1 complex in cells[57]. Similarly for gene transfer-based approaches, biodistribution of viral particles may be limited by affinity to the cell surface or extracellular components.

For growth factor-based therapies, it should be considered whether continuous presence of the therapeutic is required. Target engagement in a pulsatile fashion may have benefits over continuous infusion. For example, ligand-dependent receptor downregulation and desensitization is a key physiological mechanism that has evolved to protect cells from overstimulation[58]. Receptor desensitization that may occur during continuous administration of growth factors could decrease responsiveness of the target tissue to the therapeutic. Intermittent delivery may also offer other benefits, such as reduced risk of loss of protein potency during long incubation period in implanted infusion pumps and reduced risk of pump malfunction and better monitoring of the infusion process and parameters. There has been significant progress in development of drug delivery systems for intermittent intracranial administration of biopharmaceuticals, liposomes, viruses and cells[59,60]. Significant progress has been made in the optimization of the infusion protocol, individual device components and neurosurgical techniques[61,62]. This provides an opportunity to start assessing the pharmacodynamic properties of neurorestorative biopharmaceuticals with significantly reduced risk of failed drug delivery to the target tissue.

Intracranial delivery of therapeutics poses additional risks for the patient. All studies involving invasive intracranial procedures have reported adverse effects related to the drug delivery device or procedure, such as headache, local swelling, and skin reactions. Aside from procedural adverse effects, mild-to-moderate side effects that were likely associated with investigational therapeutic were also reported. The central side effects of GDNF varied depending on the route of administration. Appetite suppression, nausea, vomiting, and weight loss was observed in the GDNF study using i.c.v. administration[15], but not in the GDNF studies using intraputamenal administration[16,17]. In the first open-label GDNF study, mild intermittent Lhermitte's phenomenon, a tingling passing from the neck down the arms and legs provoked by neck flexion, was reported by patients[16]. In addition, high signal intensity in T2 magnetic resonance images (MRIs) around the tips of catheters was found. While the cause for these MRI findings remains unclear, the authors speculated that this could be related to vasogenic edema or protein buildup near the catheter tip. In addition, in the second open-label GDNF study some patients experienced sporadic Lhermitte's phenomenon and similar T2 MRI findings around the catheters were reported[17]. Overall, intraputamenally administered GDNF[16,17] and i.c.v. administered PDGF-BB[39] were found to be safe and well-tolerated. Similarly, in the first growth factor gene therapy clinical study in PD patients, intraputamenal NRTN gene therapy was found to be safe and well tolerated[29]. Out of 12 patients in this study, 3 reported on-medication dyskinesias, and 1 patient had hallucinations that were possibly related to CERE-120. Some patients had asymptomatic serum antibody responses to AAV2 but no evidence of viral shedding was found. T2 MRI signal changes seen postoperatively along the trajectory path of the needle were considered to be associated with the surgical procedure[29]. Clearly, clinical studies completed so far suggest that biggest safety concerns for clinical use of intracranial growth factor therapies relate to the drug delivery device and the invasive implantation procedure.

One common challenge for intracerebral drug therapies is the stage of patients to be treated. In order for the growth factor-based therapies to work, some neurons with synaptic contacts in the caudate putamen should be left to be rescued. On the other hand, patients at a very early stage of the disease should not be exposed to the risks of invasive procedures like surgical implantation of a drug delivery device. Nigral neuron counts and striatal dopamine levels are estimated to be diminished by 50% and 80%, respectively, by the time of PD diagnosis[63,64], and the gradual loss continues so that the loss of integrity of the nigrostriatal pathway, based on the rate of dopaminergic marker loss, is nearly complete by 4–5 years after diagnosis[34]. Thus, the earlier the treatment can be started, the better efficacy can be expected for any NTF-based therapy, as was clearly shown by the NRTN clinical trials[32]. However, the risk of misdiagnosis, particularly with atypical Parkinsonian syndromes, in early-stage PD patients is remarkably high[65]. Thus, careful consideration, from both clinical and ethical perspectives, is required.

CDNF is an Unconventional Neurotrophic Factor

Due to the molecular properties of GDNF and NRTN, which limit their biodistribution following intraparenchymal infusion, there is interest in novel molecules that have significant neuroprotective and neurorestorative effects on the nigrostriatal pathway but biophysical properties better suited for intracranial delivery. The two most recently discovered proteins with neurotrophic factor-like activity and that have such potential are cerebral dopamine neurotrophic factor (CDNF)[66] and mesencephalic astrocytic neurotrophic factor (MANF)[67].

Mechanistic Implications from Protein Structure

Although CDNF was originally discovered and described as a neurotrophic factor, the current view is that CDNF (and MANF) are structurally (Fig 1) and functionally (Fig 2) distinct from the classical NTF[68]. CDNF is a conserved protein in vertebrates and invertebrates, and shares no significant sequence homology to other proteins with the exception of MANF[66]. CDNF and MANF are small monomeric proteins with a molecular weight of approximately 18 kDa (mature proteins 161 and 158 amino acids, respectively) that are expressed in the central nervous system but also in non-neuronal tissues. They contain an N-terminal signal peptide that directs them to the ER. Notably, both CDNF and MANF also contain a C-terminal KDEL-like ER-retention signal

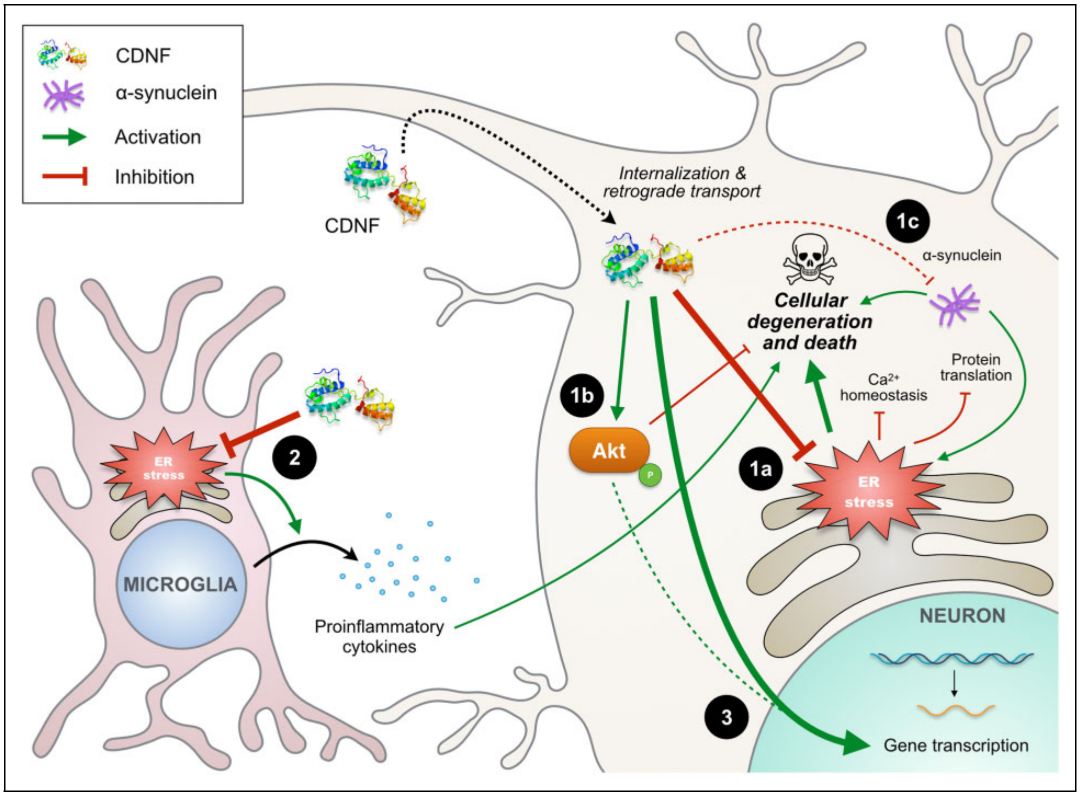

Fig 2. CDNF protects and improves functionality of stressed neurons via multiple mechanisms. (1a) CDNF suppresses chronic ER stress through modulation of UPR pathways. Global suppression of translation and altered Ca^{2+} homeostasis are among the consequences of prolonged ER stress in neurons, both known to impair synaptic function. CDNF helps to finetune UPR signaling towards adaptive stress signaling, reducing cell death and improving neuronal functionality. (**1b**) CDNF promotes the activity of Akt/protein kinase B further supporting neuronal survival. (**1c**) CDNF interferes with α-synuclein oligomerization and toxicity. (**2**) Chronic ER stress promotes neuroinflammation which exacerbates neuronal dysfunction. CDNF suppresses neuroinflammation by reducing synthesis and secretion of proinflammatory cytokines by microglial cells. (**3**) Exogenously administered CDNF has long-term effects in the brain. These effects are likely mediated by altered gene transcription.

that is typically absent in growth factors destined for secretion. Both CDNF and MANF accumulate in the ER lumen in healthy cells and disruption of the C-terminal ER-retention signal results in their secretion[69–71]. Detectable levels of CDNF and MANF are found in normal human serum, and MANF also in cerebrospinal fluid (CSF)[72]. CDNF has two potential N-glycosylation sites but glycosylation is not required for neuroprotective activity of the protein[66,73]. Neither protein is glycosylated when expressed in mammalian cell lines.

Although CDNF and MANF share only $\sim$60% amino acid sequence homology, they have highly similar three-dimensional structures[74,46]. The structure of CDNF is composed of two independently folded domains connected by a flexible loop region (Fig 1D). The secondary structure is predominantly α-helical, with five α-helices in the N-terminal domain, and three α-helices in the C-terminal domain. Three disulfide bridges stabilize the N-terminal domain while the C-terminal CRAC sequence forms an internal disulfide bridge. This CXXC disulfide bridge is found both in CDNF and MANF and it is similar to CXXC motifs found in oxidoreductases and disulfide isomerases[75]. Analysis of the C-terminal CXXC motif of MANF did not find evidence of oxidoreductase activity but showed that the CXXC motif is essential for the neuroprotective activity of MANF[76,77]. Although similar analysis has not been published for CDNF, based on the structural and functional similarities between CDNF and MANF, it would seem reasonable that the CXXC motif would be essential for the neuroprotective activity of CDNF as well.

Despite extensive research efforts proteinaceous cell surface receptors for CDNF and MANF have not been identified. Henderson et al suggested that cell surface localized KDEL receptors, translocated to cell surface in ER-stressed cells, could mediate cell surface binding of MANF (and possibly also CDNF)[70]. It is also possible that lipid-mediated interactions with the cell surface could play a role[78]. The structures of the N-terminal domains of CDNF and MANF have a typical globular saposin-like architecture[74]. Saposins are cysteine-rich proteins that interact with lipids and membranes. Thus, owing to the saposin-like structure of the N-termini, it seems plausible that lipid-binding could mediate the initial cellular interaction and internalization of these unconventional NTF. Supporting this view, binding to sulfatide, also known as 3-O-sulfogalactosylceramide, was recently suggested to mediate internalization and cytoprotective effects of extracellular MANF[79]. Further lipid interactomics studies may provide important new information on the cytoprotective mechanisms of CDNF and MANF.

Endoplasmic Reticulum as the Main Site of Action

Different from classical NTFs, CDNF and MANF can protect cells as intracellular proteins but have no effects when added to the media in healthy cultured neurons[66,76,77]. However, they have potent neuroprotective effects when infused to the brain parenchyma of lesioned animals or microinjected into lesioned neurons[66,76,80,81]. Intrastriatally infused CDNF protein is internalized by cortical and striatal neurons, primarily by dopamine neurons, and is retrogradely transported to the substantia nigra[81,82]. Electron microscopy showed that CDNF localized to endosomes and multivesicular bodies in neurons after intraparenchymal infusion. It remains currently unknown if and how internalized exogenous CDNF is transported to the ER. Notably, the responsiveness of cultured cells to extracellular CDNF and MANF can be increased by exposing the cells to various stressors, such as MPP+, rotenone, tunicamycin, and thapsigargin. Thus, CDNF appears to have potent effects on stressed or injured neurons but has no or little effect on healthy cells[76,81,83]. This is an important property when considering potential side effects in therapeutic use in humans.

The preferred localization to the ER lumen and regulated secretion of endogenous CDNF and MANF suggests they may be involved in regulation of ER function and homeostasis, and possibly serving as secreted paracrine regulators of stress response in specific tissues. A particularly important homeostatic cellular signaling system located at the ER is the unfolded protein response (UPR) pathway[84]. The ER is an important stress-sensing and regulating organelle in cells, and the UPR serves as a dynamic and adaptive signaling system in the ER helping to restore cellular homeostasis during ER stress. ER stress has been increasingly recognized as a general mechanism involved in a broad variety of human diseases[85,86]. The pathophysiology of many chronic diseases, in particular neurodegenerative diseases, has been shown to involve the UPR pathway and chronic ER stress[86–88].

The three main signaling arms of the UPR are triggered in mammalian cells by activation of PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1α (IRE1α) located at the ER membrane[84]. As ER stress attenuates general protein translation, synapses are likely very sensitive to prolonged ER stress[89]. Long-lasting forms of synaptic plasticity are highly dependent on protein synthesis[90]. Recent evidence suggests that the UPR and the ER proteostasis network are fundamentally involved in the maintenance of neuronal physiology at multiple levels of synaptic function and connectivity[91]. Prolonged or severe ER stress can trigger cell death via the pro-apoptotic mode of UPR[92].

In PD, there are multiple lines of evidence linking UPR to several disease-relevant pathways (reviewed by Mercado et al[93]). Post-mortem analysis of PD brain tissue revealed abnormally phosphorylated PERK, IRE1, and eukaryotic translation initiation factor 2α (eIF2α) in dopamine neurons of the SNpc, and activated PERK and IRE1 colocalizing in neurons with α-synuclein inclusions[94–96], which are the main component of Lewy bodies—a neuropathological hallmark of PD. Triplication of the α-synuclein-encoding SNCA gene in iPS cell-derived neurons, mimicking cellular pathology of early-onset PD, resulted in induction of the IRE1/XBP1 axis of the UPR and increased expression of pro-apoptotic UPR target genes CHOP and BIM[95]. Expression of ER stress-related proteins, including GRP78/BiP, XBP1, CHOP, and ATF4, is also increased in the brain of α-synuclein transgenic mice, and the presence of toxic α-synuclein oligomers at the ER correlates with elevated level of ER stress and faster disease progression *in vivo*[97,98]. The ER may serve as a potential site of accumulation of toxic α-synuclein conformers[99], and accumulation of misfolded α-synuclein in the ER has been reported in brain tissue from human PD patients[100]. Pathological forms of α-synuclein may induce ER stress by directly altering the ER proteostasis or indirectly by impairing ER-to-Golgi traffic[101] or by altering ER Ca^{2+} homeostasis[102]. On the other hand, ER stress was shown to promote α-synuclein aggregation providing a feedback loop between ER stress and α-synuclein aggregation[103]. Mutant forms of α-synuclein (A53 T and A30P), found in familial forms of PD, were shown to trigger ER stress also in astrocytes via the PERK-eIF2α pathway resulting in reduced GDNF secretion and increased astrocyte apoptosis[104], which likely contributes to PD pathogenesis.

Interestingly, many genes associated with PD can modulate the function and stress responses of the ER. ER stress regulates both expression and subcellular distribution of Parkin/PARK2[105,106]. Expression of Parkin-associated endothelin receptor-like receptor Pael-R, a substrate of Parkin ubiquitin ligase, induces ER stress and neurodegeneration in the SNpc of mice[107]. Downregulation of DJ-1/PARK7 enhances the susceptibility of cells to ER stress and cell death[108]. Although direct mechanistic evidence is still lacking, dysregulated function of leucine-rich repeat kinase 2 (LRRK2), PTEN-induced kinase 1 (PINK1) and glucocerebrosidase (GBA1) have also been linked to altered ER stress responses *in vivo*[109–111].

Toxin-based models of PD (MPTP, 6-OHDA, and rotenone) show prominent activation of the PERK and IRE1α pathways[112,113]. Several studies have shown that targeting the UPR pathway genetically can robustly alter the course of dopamine neuron loss following 6-OHDA or MPTP lesioning[114,115]. Moreover, daily administration of GSK2606414, an orally available PERK inhibitor, to 6-OHDA lesioned mice for 3 weeks resulted in strong neuroprotection of dopamine neurons, increased striatal dopamine levels and improved motor performance[96]. Thus, the toxin-based models of PD are well suited for studying the therapeutic potential of ER stress modulating compounds. Notably, when CDNF and GDNF were compared in the 6-OHDA model of PD, both proteins activated the survival promoting PI3K-Akt signaling pathway, but only CDNF decreased the expression level of tested ER stress markers ATF6, GRP78, and phosphorylation of eIF2α[116].

Neuroinflammation—a key component of most if not all neurodegenerative diseases—is induced by ER stress[117,118]. Brain-resident microglia and astrocytes are the main source of inflammation in diseases affecting the brain. In glial cells, both PERK-eIF2α and IRE-1α-TRAF2-IKK pathways can activate NF-κB, a central regulator of multiple aspects of immune functions[119]. PERK pathway can also promote STAT3 signaling via JAK1[120] while the IRE1α pathway can activate both JNK and p38 kinases via ASK1[121,122]. There are thus multiple ways ER stress can promote inflammatory responses in glial cells.

In vitro data shows that CDNF expression is induced by ER stress in cultured neurons[123], and that CDNF expression improves neuronal viability by upregulating several proteins involved in UPR signaling, including GRP78, ATF4, ATF6, and XBP1, while reducing activation of ER stress-responsive apoptotic proteins, such as CHOP[124]. Similarly, CDNF overexpression in cultured astrocytes alleviated ER stress-induced cell damage and reduced secretion of proinflammatory cytokines[125]. Moreover, in cultured microglial cells, CDNF reduces lipopolysaccharide-induced, JNK-mediated secretion of proinflammatory cytokines PGE2 and IL-1β[126]. Transient expression of CDNF in the SN was also shown to reduce markers of nitrosative stress and level of IL-6 after 6-OHDA lesioning[127], suggesting that alleviation of neuroinflammation contributes to the therapeutic effects of CDNF in the toxin-based models of PD.

In the invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster*, genetic disruption of the single CDNF/MANF ortholog resulted in degeneration of dopamine neurons linked to dysfunction of the ER and elevated ER stress level[128,129]. MANF-deficient mice strikingly develop severe diabetes due to progressive postnatal apoptosis of pancreatic β cells associated with chronic UPR activation[130]. Interestingly, CDNF-deficient mice display an enteric nervous system phenotype relevant to gastrointestinal non-motor symptoms of PD[131].

Finally, in a recent study, MANF was shown to bind to the nucleotide-binding domain of GRP78 and inhibit both ADP release from GRP78 and ATP binding to GRP78, suggesting that MANF contributes to protein folding homeostasis as a nucleotide exchange inhibitor that stabilizes certain GRP78-client complexes[132]. Although a similar interaction has not been shown for CDNF, it seems plausible that both CDNF and MANF would be involved in regulation of ER homeostasis via direct protein–protein interactions with some of the key molecules in the ER lumen, such as GRP78 chaperone.

Collectively, the structural and mechanistic studies strongly suggest that CDNF and MANF are primarily ER lumen-located proteins with potent cytoprotective properties in multiple cell types and tissues. CDNF and MANF can be secreted, likely related to cellular stress, and can protect neighboring cells in a paracrine fashion. The cell-based mechanistic studies are supported by phenotypes of CDNF and MANF knockout animals suggesting that these proteins are intimately linked with the regulation of UPR signaling and cellular tolerance to ER stress. Despite the *in vivo* neuroprotective properties of CDNF and MANF, their basic biological properties clearly suggest that they should not be classified as conventional NTF. They are rather ER-located proteins with unconventional neurotrophic activities. Currently available data on cellular mechanisms and pathways regulated by CDNF in neuronal and glial cells are summarized in Fig 2.

Preclinical Pharmacology and Toxicology Studies

Single unilateral intrastriatal injection of CDNF protein in the rat 6-OHDA model of PD, both before and after lesioning, resulted in robust recovery of motor functions, and protection and regeneration of tyrosine hydroxylase (TH)-positive dopamine neurons and their fibers in the nigrostriatal pathway[66]. Similarly, 2-week chronic intrastriatal infusion of CDNF via implanted osmotic minipumps gradually normalized the motor behavior of the 6-OHDA lesioned rats, with prominent regeneration and sprouting of TH-positive fibers in the nigrostriatal pathway while GDNF in comparison had only modest effects[81]. Notably, CDNF showed a significantly larger volume of diffusion in this study compared with GDNF. Two studies found that viral (AAV2) expression of CDNF in the striatum protected from 6-OHDA induced impairment of motor function with partial protection of TH-positive cells in the SNpc and TH-positive fibers in the striatum[31,133]. Another study suggested that combined nigral delivery of lentiviral CDNF and MANF provided stronger protection of nigral dopamine neurons and increased TH+ fiber density in striatum compared to individual proteins[134]. However, it is rather difficult to draw final conclusions from this study, because the biological activities of the CDNF and MANF proteins produced by the respective lentiviral vectors, was not reported. Furthermore, the levels of CDNF and MANF in the midbrain after gene therapy remained also unclear.

In MPTP-lesioned mice, bilateral striatal CDNF injections, given either 20 h before or 1 week after MPTP, improved horizontal and vertical motor behavior and increased TH-immunoreactivity in the striatum and the number of TH-positive cells in SNpc[83].

As expected, the therapeutic effects of CDNF are dependent on the number of remaining dopamine neurons in the nigrostriatal pathway. In the 6-OHDA-based major forebrain bundle (MFB) lesion model, mimicking late-stage PD-like loss of nigrostriatal DAergic function, intranigral CDNF injections had only marginal effect on motor function[135]. However, CDNF injection improved the effect of acute subthalamic deep brain stimulation (DBS) on front limb use asymmetry at 2 and 3 weeks after CDNF injection and increased the density of striatal TH staining[135]. This suggests that CDNF therapy and DBS could have additive therapeutic effects in PD patients.

Notably, GDNF failed to exert neuroprotection in a rodent model of PD based on viral vector-expressed α-synuclein[136,137] despite prominent efficacy in toxin-based models. In rats, strong overexpression of human α-synuclein was reported to drastically reduce the amount of Nurr1 and consequently the levels of GDNF receptor Ret protein[138]. This study is, however, under debate[139]. As α-synuclein accumulation is frequently observed in PD patients, it was suggested that the loss of Ret might partially explain the lack of efficacy of GDNF in the previous clinical trials[138]. In a recent study, Su et al evaluated the expression levels of α-synuclein and GDNF signaling molecules (e.g., Ret and Nurr1) in PD patient brain samples, α-synuclein transgenic mice, and AAV-α-synuclein injected rats[139]. They found that α-synuclein mRNA is not increased in sporadic PD and accumulation of α-synuclein does not suppress the expression of GDNF signaling molecules, including its receptor Ret, in PD patient samples, and disease models.

Owing to the central role of α-synuclein in the pathogenesis of PD, it is important that novel PD therapeutics are tested also in α-synuclein-based models. CDNF was shown to protect dopamine neurons from α-synuclein oligomer toxicity *in vitro* [46], but efficacy in α-synuclein-based animal models still remains unpublished.

Non-human primate (NHP) studies have been conducted with intrastriatally infused CDNF. In 6-OHDA lesioned marmoset monkeys (*Callithrix jacchus*), PET imaging showed a significant increase of DAT ligand binding activity in lesioned animals treated with CDNF[140]. In addition, CDNF has been tested in a unilateral MPTP model in aged Rhesus macaques (*Macaca mulatta*)[141].

Neuroprotective and neuroregenerative effects of CDNF are not specific to the dopaminergic system and various positive therapeutic outcomes have been reported in other models of neurodegeneration, including contusion spinal cord injury in rats[142], rat middle cerebral artery occlusion (MCAO) model of cerebral ischemia[123], APP/PS1 mouse model of Alzheimer's disease[143], and genetic models of amyotrophic lateral sclerosis (ALS)[144].

In a single dose pharmacokinetics study in healthy rats, CDNF was bilaterally infused into the rat striatum at two dose levels. The pharmacokinetic profile was similar for both doses, the tissue half-life of CDNF protein in the striatum being 5.5 h and in the substantia nigra approximately 9 h. In a pilot toxicology study in Rhesus macaques (*M. mulatta*), toxicokinetic samples of CSF and plasma were obtained at different timepoints post-first-intraputamenal dosing of CDNF. The individual variability was rather large, with average peak plasma levels at 15 min. At 24 h, the plasma levels were below the limit of detection in all animals. The highest peak CSF concentration measured roughly 300-fold the CDNF level in plasma. At 72 h, the CSF levels were reduced significantly, although still measurable. In the main, non-human primate toxicology study, a primary peak of absorption was observed at <0.5 h following the end of infusion. Following a single infusion, concentrations of CDNF in CSF were generally only detected 4 h following the end of infusion, and were generally higher when compared with Cmax in plasma[145].

Toxicology studies based on repeated (monthly) bilateral intraputamenal infusions of CDNF were performed in Rhesus macaques as NHPs are the most relevant species based on *in vivo* pharmacologic activity and anatomical comparability with the human brain structure. The toxicology program was composed of three studies: a maximum feasible dose tolerability study ($n = 8$), a 3-month pilot repeat dose toxicology study (GLP; $n = 8$), and a 6-month repeat dose toxicology study with a 4-month recovery phase (GLP; $n = 36$). Intraputamenal infusion of CDNF to male and female Rhesus macaques was well-tolerated with no CDNF-related clinical signs, body weight/food consumption effects, alterations in clinical pathology parameters (haematology, coagulation, clinical chemistry, or urinalysis parameters), electrocardiogram (ECGs), blood pressure, effects on ophthalmological or neurological evaluations, gross tissue evaluations or organ weights, nor were there any macroscopic or microscopic changes observed in histopathological examination (Herantis Pharma Plc, unpublished data). No specific genotoxicity/mutagenicity, carcinogenicity or reproductive toxicology studies have been conducted with intraputamenally infused CDNF. The human equivalent dose of the highest dose of CDNF in the main toxicology study has a 68-fold safety factor to the first-in-human dose of 120 μg, and a 7-fold safety factor to the highest clinical dose of 1200 μg used in the phase I–II clinical study.

Phase I–II Clinical Study of Intraputamenal CDNF in PD

The first-in-human study with CDNF, sponsored by Herantis Pharma Plc, was started in three centers in Sweden and Finland in late 2017. In this randomized, placebo-controlled, interventional, multi-center, phase I–II study, 18 patients with idiopathic moderately advanced PD (bilateral, Hoehn and Yahr ≤3, disease duration ≥5 years) will be enrolled (ClinicalTrials.gov identifier NCT03295786). Monthly infusions of CDNF at three ascending dose levels will be given for 6 months in the randomized, placebo-controlled main study followed by an extension study in which all patients will receive CDNF. A neurosurgically implanted drug delivery system essentially similar to the one used in the Bristol GDNF phase II study will be used for intermittent intraputamenal delivery of CDNF[61,62]. The investigational medicinal product in this study is recombinant human CDNF protein manufactured in a mammalian cell line. The biological activity and stability of the protein and its compatibility with the drug delivery device system has been carefully tested[145].

The primary endpoint of the study is safety and tolerability of intraputamenal CDNF with a co-primary endpoint assessing safety and implantation accuracy of the drug delivery system. Secondary objectives include, e.g., evaluation of drug effects on PD symptoms by UPDRS (part III), timed up and go test, activities of daily living (UPDRS part I-IV), patient home diary, PD Questionnaire-39 and Clinical Global Scale (CGI). An important exploratory objective of the study is assessment of the change in caudate and putamen DAT availability using PET imaging with [^{18}F]FE-PE2I to assess the integrity of the nigrostriatal system[146,147]. Other exploratory endpoints include serum and CSF levels of total α-synuclein, oligomeric α-synuclein and serine-129 phosphorylated α-synuclein[148], the level of distribution of CDNF in serum and CSF after infusion, and periodical assessment of motor complications by Parkinson's KinetiGraph™ (PKG™) data logger[51].

Future Perspectives

As there is preliminary clinical evidence that growth factor-based treatments have disease-modifying effects in PD patients, there is a continued motivation to develop improved biopharmaceuticals and drug delivery methods in order to slow down, or even stop, the progression of this chronic debilitating disease. Development of growth factor-based therapies for PD has suffered from drug delivery challenges. Novel drug delivery devices and protocols have been developed to address these issues. In addition, novel proteins with neurotrophic activity, such as CDNF, have been discovered and developed to the clinical stage. CDNF has a unique mode of action (regulation of UPR, prevention of apoptosis, and reduction of glial secretion of proinflammatory cytokines) targeting preferentially injured cells, which clearly differentiates it from conventional NTFs. Brain-infused CDNF also diffuses broadly in the tissue, particularly in comparison to GDNF and NRTN, which may result in additional benefits beyond the nigrostriatal pathway, with potential effects on some of the non-motor symptoms of PD. There is an indication of disease modification from the NRTN gene therapy trials, and PET imaging has indicated restorative effects on the nigrostriatal pathway in humans both with GDNF and NRTN. These important findings encourage further development of disease-modifying therapies—a major unmet clinical need in PD—based on NTFs and other molecules with neuroprotective and neuroregenerative properties.

For improving translational success of growth factor-based, potentially disease-modifying therapies in PD, preclinical studies should carefully mimic the clinical application, particularly regarding drug delivery. In addition to toxin-based models using old animals, α-synuclein-based models, as well as patient iPS cell-derived human dopamine neurons should be included in the preclinical program. Previous clinical studies have shown that effective delivery to, and distribution within, the target tissue is essential for clinical efficacy. The challenge of accessing earlier stage PD patients with therapeutics that require invasive procedures has to be resolved. As growth factor-based therapies aim mostly at protection and functional restoration of the remaining neurons in the nigrostriatal pathway, the stage of patients enrolled to efficacy studies will be critical. Clearly, the longer the disease has progressed before the treatment is started the less neurons there are to protect and recover, and the lower the chances are for meaningful clinical improvement. On the other hand, the risk of misdiagnosis with earlier stage patients and ethical concerns of invasive procedures cannot be ignored. Development of growth factor-action mimicking small molecules that are suited for peripheral administration in earlier stage patients could help to overcome many of the challenges related to invasive drug delivery.

Improved understanding of disease subtypes and mechanisms of disease progression in PD should guide clinical study designs. As PD is known to be etiologically heterogeneous, clinical study designs with homogenous patient populations is expected to increase the odds of success. Properly powered studies with placebo control groups with delayed start design should be preferred over small open-label studies. Surrogate biomarkers, such as PET imaging to assess the integrity of the nigrostriatal pathway, will play an important role in establishing clinical proof-of-concept, as the clinical rating scales are not well suited for smaller trials. Furthermore, progress with biomarker research together with the advent of wearable digital technologies will provide more sensitive and more objective assessments and endpoints for clinical studies testing novel disease-modifying therapies in PD. Last, but not least, due to the immense complexity of the human brain, patience and persistence is needed from sponsors, patients, investors and other stakeholders in the process of developing disease-modifying therapies for PD.

Ethical Approval

Ethical Approval is not applicable for this article.

Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: HJH is an employee, and both HJH and MS are shareholders of Herantis Pharma Plc. Herantis Pharma develops rhCDNF for Parkinson's disease.

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