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Enhanced neurotrophic distribution, cell signaling and neuroprotection following substantia nigral versus striatal delivery of AAV2-NRTN (CERE-120)

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ABSTRACT

This paper reassesses the currently accepted viewpoint that targeting the terminal fields (i.e. striatum) of degenerating nigrostriatal dopamine neurons with neurotrophic factors in Parkinson's disease (PD) is sufficient for achieving an optimal neurotrophic response. Recent insight indicating that PD is an axonopathy characterized by axonal transport deficits prompted this effort. We tested whether a significantly greater neurotrophic response might be induced in SN neurons when the neurotrophic factor neurturin (NRTN) is also targeted to the substantia nigra (SN), compared to the more conventional, striatum-only target. While recognizing the importance of maintaining the integrity of nigrostriatal fibers and terminals (especially for achieving optimal function), we refocused attention to the fate of SN neurons. Under conditions of axonal degeneration and neuronal transport deficits, this component of the nigrostriatal system is most vulnerable to the lack of neurotrophic exposure following striatal-only delivery. Given the location of repair genes induced by neurotrophic factors, achieving adequate neurotrophic exposure to the SN neurons is essential for an optimal neurotrophic response, while the survival of these neurons is essential to the very survival of the fibers. Two separate studies were performed using the 6-OHDA model of nigrostriatal degeneration, in conjunction with delivery of the viral vector AAV2-NRTN (CERE-120) to continuously express NRTN to either striatum or nigra alone or combined striatal/nigral exposure, including conditions of ongoing axonopathy. These studies provide additional insight for reinterpreting past animal neurotrophic/6-OHDA studies conducted under conditions where axon transport deficiencies were generally not accounted for, which suggested that targeting the striatum was both necessary and sufficient. The current data demonstrate that delivering NRTN directly to the SN produces 1) expanded NRTN distribution within the terminal field and cell bodies of targeted nigrostriatal neurons, 2) enhanced intracellular neurotrophic factor signaling in the nigrostriatal neurons, and 3) produced greater numbers of surviving dopamine neurons against 6-OHDA-induced toxicity, particularly under the conditions of active axonopathy. Thus, these data provide empirical support that targeting the SN with neurotrophic factors (in addition to striatum) may help enhance the neurotrophic response in midbrain neurons, particularly under conditions of active neurodegeneration which occurs in PD patients.
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Introduction

Significant evidence demonstrates that, like many neurodegenerative diseases, Parkinson's disease (PD) progresses as an axonopathy (Burke, 2010; Cheng et al., 2010; Hilliard, 2009) and that deficits in axonal transport occur as important and relatively early pathologic events (Chu et al., 2012; De Vos et al., 2008; Morfini et al., 2009; O'Malley, 2010; Raff et al., 2002; Roy et al., 2005). The lack of proper axonal transport not only can interfere with many other neuronal functions crucial for trans-synaptic communication, but may also reduce the effectiveness of interventional therapeutics, such as those using neurotrophic factors intended to repair the degenerating neurons (e.g., see Bartus et al., 2011a). Brain autopsy data from two PD subjects who died from unrelated causes 1.5 and 3 months following intrastriatal gene transfer of human neurturin (via AAV-NRTN or CERE-120) confirmed NRTN expression in the terminal fields (i.e.,

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the targeted putamen) of dopaminergic neurons projecting from the substantia nigra (SN). However, unexpectedly, only sparse evidence for NRTN was seen in the degenerating nigrostriatal perikarya, located in the SN pars compacta (SNc) (Bartus et al., 2011a). These data potentially explained the somewhat disappointing, mixed results of that clinical trial, where the primary endpoint (UPDRS, Motor off) did not show benefit at 12 months, despite several other clinically important, prescribed endpoints showing statistically significant improvement. Moreover, an apparent augmentation in clinical benefit was seen over time, with several more endpoints improving at 18 months, including the primary endpoint (and no endpoint similarly favoring sham at either time point) (Bartus, 2012; Bartus et al., 2013; Marks et al., 2010). Without sufficient transport of NRTN from the putamen (where the protein was expressed) to the SNc, induction of repair genes in the nuclei of the SNc dopamine neurons is far less likely to occur, producing a suboptimal neurotrophic response. Consistent with this hypothesis, tyrosine hydroxylase (TH) induction in the putamen (i.e., a well-accepted surrogate for enhanced status of degenerating dopamine neurons) in the same CERE-120 PD cases was modest and far less robust than occurs following CERE-120 administration to young adult, aged or MPTP-treated nonhuman primate models with similar or even less NRTN coverage in the striatum (Bartus et al., 2011a).

These observations, along with the accumulating evidence that axonal transport deficits occur as an early pathogenic event in PD, suggest that achieving optimal benefit from neurotrophic factors in advanced PD likely requires targeting both the cell bodies in the SN and the terminal fields in the striatum (Bartus, 2012). However, this idea runs counter to contemporary consensus that argues it is sufficient to only target the terminal field (i.e., striatum) with the neurotrophic factor (Ciesielska et al., 2011; Kells et al., 2012; Kirik et al., 2000a, 2000b, 2004). Indeed, all published clinical efforts to date, have presumed that targeting the terminal fields should be sufficient (Gill et al., 2003; Lang et al., 2006; Marks et al., 2008, 2010; Slevin et al., 2005). However, the animal data supporting this supposition were generated without the benefit of the more recent insight that significant axonal transport deficiencies can negatively impact the bioactivity of neurotrophic factors in PD when delivered solely to their terminal fields. Thus, the extensive animal literature generated over the past decade may not adequately inform us about the most appropriate dosing paradigms or anatomical targets for advanced PD as much as we and the vast majority of investigators in the field had all come to presume (Bartus, 2012; Gill et al., 2003; Lang et al., 2006; Marks et al., 2008, 2010; Slevin et al., 2005).

Indeed, despite relatively recent, notable exceptions (Chung et al., 2009; Decressac et al., 2012a, 2012b; Kirik et al., 2002), the most widely used animal models to induce degeneration of nigrostriatal neurons still involve the use of acute dosage regimens of neurotoxins (e.g, MPTP and 6-OHDA) in conjunction with one of two main dosing paradigms. These involve either (1) a 'neuroprotection paradigm', where the neurotrophic factor is administered well in advance of the neurotoxin, or (2) a 'neurorestoration' paradigm that delivers neurotrophic factors well after the neurotoxin (typically several weeks later). In this latter case, by the time the neurotrophic factor is delivered, the neurotoxin has been cleared (i.e., metabolized), the denervation of dopamine terminals to the striatum is mostly complete, and those neurons that have survived the acute neurotoxin perturbation are now in the process of recovery. In other words, because much of the prior animal research did not appreciate the profound axonal transport deficiencies that occur as early-stage events in axonopathies like PD, or the impact they might have on the efficacy of certain treatment approaches, the paradigms employed to evaluate targeting options may have been suboptimal for defining the most appropriate dosing paradigm to use in the clinic where such axonopathies are prevalent (De Vos et al., 2008; Morfini et al., 2009; Raff et al., 2002; Roy et al., 2005), which includes patients with moderate to advanced PD (Chu et al., 2012; O'Malley, 2010).

The major question addressed in this paper is whether, in the midst of ongoing nigrostriatal degeneration, a more robust neurotrophic response might be achieved by directly targeting the SN with the neurotrophic factor. While the preservation of nigrostriatal dopaminergic fibers has been shown to be important for functional benefit, and targeting these fibers directly improves their survival and viability, it is also certainly true that preservation of SN neurons is required for the preservation of those fibers. Additionally, optimizing a neurotrophic response also requires assurance that the SN neurons are exposed to elevated levels of the neurotrophic factor. The major reason that this issue has not drawn attention in the past is because virtually all studies targeting the striatum did so under conditions where retrograde transport mechanisms from the striatal to the SN were still sufficiently intact and thus the SN was concurrently exposed via retrograde transport to the neurotrophic factor following striatal administration. However, in the midst of active axonal degeneration and transport deficiencies, these neurons may be vulnerable to substantially reduced exposure to the neurotrophic factor administered to the striatum-only, thus impeding an optimal neurotrophic response.

In an effort to more directly test whether SN targeting of neurotrophic factors might possibly provide a greater neurotrophic factor response under conditions of axonal dysfunction (as occurs in moderately-advanced PD subjects), beyond that provided by striatal targeting, we conducted two separate experiments in rats. The first experiment attempted to evaluate the effects of NRTN expression under conditions that more closely mimic degenerating PD nigrostriatal neurons actively undergoing axonopathy. This was accomplished by employing a previously characterized 6-OHDA model of progressive degeneration that, following striatal delivery of the toxin, results in an initial "dying back" or axonopathy of dopaminergic terminals over a period of several weeks, with eventual death of dopaminergic nigral neurons occurring as the end-stage event (Blandini et al., 2007; Lee et al., 1996; Sauer and Oertel, 1994; Walsh et al., 2011). When the gradual dying back of nigrostriatal fibers following appropriate doses of striatal-6-OHDA is considered together with the gradual ramp-up time for full NRTN expression following CERE-120 (AAV2-CAG-NGFprepro-NRTN) injections into brain (Gasmi et al., 2007a), the simultaneous administration of 6-OHDA and CERE-120 ensures that NRTN expression in the targeted regions would gradually increase as the nigrostriatal dopamine neurons were in the midst of progressive degeneration and axonopathy.

The second experiment attempts to re-examine the question of whether adding SN targeting to an optimal dose of striatal targeting of a neurotrophic factor might provide any additional neurotrophic advantage. For this study, we used the traditional "neuroprotection" paradigm. Because that paradigm is arranged so that NRTN is expressed and present well in advance of toxin administration it is delivered well before axonopathy is initiated, providing ample time for the still-healthy, unperturbed neurons to transport NRTN from the targeted striatum to the SN and initiate a trophic response. Thus, while not ideal for predicting benefit in PD patients who cannot be pretreated with a neurotrophic factor, this "neuroprotection" paradigm is optimized to show a benefit from striatal-only delivery of the neurotrophic factor, therefore providing a rigorous test for whether any additional benefit might be achieved by adding SN-targeting of the neurotrophic factor. In both of these studies, CERE-120 doses for the striatum and SN were selected on the basis of prior dose–response studies which showed that each dose provided optimal efficacy against 6-OHDA following CERE-120 targeted to either the striatum (Gasmi et al., 2007b) or SN (Bartus et al., 2011b). In this way, we attempted to define therapeutic doses and provide information that would be useful for translation to the clinic. Collectively, these studies demonstrate that direct delivery of NRTN to the SN provides several advantages for potentially enhancing the neurotrophic factor response in degenerating nigrostriatal neurons. This information may have direct implications to translating a superior neurotrophic-factor-related response and therefore superior clinical benefit in PD subjects.

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