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Research Report

Application of a blood–brain-barrier-penetrating form of GDNF in a mouse model for Parkinson's disease

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ABSTRACT

Glial-cell-line-derived neurotrophic factor (GDNF) promotes mesencephalic dopaminergic neuronal survival in several in vitro and in vivo models. As the demise of dopaminergic neurons is the cause for Parkinson's disease (PD) symptoms, GDNF is a promising agent for its treatment. However, this neurotrophin is unable to cross the blood–brain barrier, which has complicated its clinical use. Therefore, ways to deliver GDNF into the central nervous system in an effective manner are needed. The HIV-1-Tat-derived cell-penetrating peptide (CPP) provides a means to deliver fusion proteins into the brain. We generated a fusion protein between the 11 amino acid CPP of Tat and the rat GDNF mature protein to deliver GDNF across the blood–brain barrier. We showed previously that Tat-GDNF enhances the neuroprotective effect of GDNF in in vivo models for nerve trauma and ischemia. Here, we tested its effect in a subchronic scheme of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) application into the mouse as a model for PD to evaluate the effect of Tat-GDNF fusion protein in dopaminergic neuron survival. We showed that the fusion protein did indeed reach the dopaminergic neurons. However, the in vivo application of Tat-GDNF did not provide neuroprotection of dopaminergic neurons, as revealed by immunohistochemistry and counting of the number of tyrosine-hydroxylase-immunoreactive neurons in the substantia nigra pars compacta. Possibly, GDNF does protect nigro-striatal projections of those neurons that survive MPTP treatment but does not increase the number of surviving dopaminergic neurons. A concomitant treatment of Tat-GDNF with an anti-apoptotic Tat-fusion protein might be beneficial.

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Abbreviations:
Tat, 11 amino acid basic domain derived from the HIV transactivator of transcription
BBB, blood–brain barrier
CPP, cell-penetrating peptide
GDNF, glial cell line-derived neurotrophic factor
HA, hemagglutinin
ip, intraperitoneally
NGS, normal goat serum
PD, Parkinson's disease
PTD, protein transduction domain
RT, room temperature
SN, substantia nigra
TH, tyrosine hydroxylase

1. Introduction

To date, only symptomatic treatments for Parkinson's disease (PD) are effective, therapies efficiently decelerating disease progression being unavailable (Koller and Cersosimo, 2004, for review). Glial-cell-line-derived neurotrophic factor (GDNF) promotes neuronal survival, specifically of dopaminergic neurons (Lin et al., 1993), those neurons affected by PD. It was therefore suggested over a decade ago that GDNF might impede disease progression (Weiss, 1993). It provided neuroprotection in vivo when injected directly into the brain, in mouse (Tomac et al., 1995), rat (Bjorklund et al., 1997), and rhesus monkey (Gash et al., 1996; Grondin et al., 2002) models for PD. The experimental procedures in these studies were invasive, making other application modes desirable. However, a molecule must be lipophilic and below 500 Da in size to directly cross the blood–brain barrier (BBB) in pharmacologically significant amounts following systemic application (Pardridge, 1998). Neurotrophins do not cross the BBB in vivo and are similar in this respect to many other potential neuroprotective agents (Pardridge, 2002, for review). Some drugs have reached the clinical trial stage, only to have been found ineffective because they did not cross the BBB or sufficiently penetrate the brain parenchyme (Dawson et al., 2001). For example, when GDNF was delivered intracerebroventricularly in clinical studies (Nutt et al., 2003; Kordower et al., 1999), it caused severe side effects without improving Parkinsonism, possibly because it did not reach its target areas in the putamen and the substantia nigra. On the other hand, in other clinical studies, direct infusion of GDNF into the putamen caused an improvement of the symptoms without serious side effects (Gill et al., 2003; Slevin et al., 2005). Even after 2 years of treatment, the patients showed a general improvement in their PD rating scale (Patel et al., 2005). In spite of these positive results, it would be desirable to apply GDNF using less invasive surgery procedures. Lentiviral gene delivery alleviated parkinsonian symptoms in rhesus monkeys (Kordower et al., 2000; Palfi et al., 2002). Although lentiviral vectors have been engineered with increased biosafety and no immune response, the psychological barrier to use viral vectors on patients remains high (Debyser, 2003).

In recent years, strategies have been developed to overcome the BBB and cellular membranes (Dietz and Bähr, 2004, 2005). One approach is to link the protein of interest to basic peptides, such as the cell-penetrating peptide (CPP) derived from the HIV Tat protein (Schwarze et al., 1999). For instance, we have generated a recombinant Tat-GNDF fusion protein purified from *E. coli* (Kilic et al., 2003; Kilic et al., 2004). The protein distributes efficiently in neuronal tissue and protects against trauma-induced cell death (Kilic et al., 2004). Moreover, systemically applied Tat-GNDF reduces infarct size after ischemia in mice (Kilic et al., 2003). As the BBB is intact for at least 3 h after stroke (Menzies et al., 1993; Belayev et al., 1996; Albayrak et al., 1997), Tat-GDNF is able to cross the BBB in sufficient amounts to protect neurons from their demise.

We wanted to test whether Tat-GNDF would also be an effective neuroprotectant in a model for PD. MPTP causes the degeneration of the nigro-striatal dopaminergic pathway and is a widely accepted experimental model for PD to date (Przedborski et al., 2004). It has since been used in PD models in primates (Langston et al., 1984) and mice (Heikkila et al., 1984). Here, we have examined whether systemically applied Tat-GDNF would protect dopaminergic neurons in the substantia nigra pars compacta against MPTP toxicity.

2. Results

2.1. *Tat-GDNF transduces the substantia nigra after intraperitoneal application*

We showed in earlier studies that systemically applied Tat-GDNF reaches all examined brain areas, including cortex, striatum, and medulla (Kilic et al., 2003). To determine whether intraperitoneally (ip) injected Tat-GDNF reaches the substantia nigra in particular, we ip-injected Tat-GDNF, or Tat-HA, which consisted of the cell-penetrating peptides (CPP) and the HA domain only, or GDNF, which did not include a Tat domain, and performed immunohistochemical analysis on the brains that were dissected 4 h after protein application. Brain sections from Tat-HA or Tat-

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