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Pharmacologically controlled, discontinuous GDNF gene therapy restores motor function in a rat model of Parkinson's disease

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ABSTRACT

Neurotrophic factors have raised hopes to be able to cure symptoms and to prevent progressive neurodegeneration in devastating neurological diseases. Gene therapy by means of viral vectors can overcome the hurdle of targeted delivery, but its current configuration is irreversible and thus much less controllable than that of classical pharmacotherapies. We thus aimed at developing a strategy allowing for both curative and controllable neurotrophic factor expression. Therefore, the short-term, intermittent and reversible expression of a neutrophic factor was evaluated for therapeutic efficacy in a slowly progressive animal model of Parkinson's disease (PD).

We demonstrate that short-term induced expression of glial cell line derived neurotrophic factor (GDNF) is sufficient to provide i) substantial protection of nigral dopaminergic neurons from degeneration and ii) restoration of dopamine supply and motor behaviour in the partial striatal 6-OHDA model PD. These neurorestorative effects of GDNF lasted several weeks beyond the time of its expression. Later on, therapeutic efficacy ceased, but was restored by a second short induction of GDNF expression, demonstrating that monthly application of the inducing drug mifepristone was sufficient to maintain neuroprotective and neurorestorative GDNF levels.

These findings suggest that forthcoming gene therapies for PD or other neurodegenerative disorders can be designed in a way that low frequency application of an approved drug can provide controllable and therapeutically efficient levels of GDNF or other neurotrophic factors. Neurotrophic factor expression can be withdrawn in case of off-target effects or sufficient clinical benefit, a feature that may eventually increase the acceptance of gene therapy for less advanced patients, which may profit better from such approaches.

Introduction

Neurodegenerative disorders like Parkinson's disease (PD) and Alzheimer's disease (AD) affect millions of patients with ever growing numbers in ageing societies, but no curative treatment exists so far. Neurotrophic factors have raised considerable hope to be able to stop the progressive degenerative phenotype (Mickiewicz and Kordower, 2011), but their appropriate delivery to target structures remains a serious issue. Gene therapy using viral vectors might overcome this delivery issue (Bartus et al., 2013), but in the current layout of gene transfer being irreversible they are currently primarily accepted for relatively advanced patients. However, in case of advanced PD patients compelling data suggest that neurotrophic factors might not be able to overcome impairments in the almost completely degenerated nigrostriatal projection (Bartus et al., 2011), an issue further illustrated by limited success of recent clinical trials exploiting the neurotrophic factor neurturin (Marks et al., 2010 and: http://www.ceregene.com/press_041913.asp). It is tempting to speculate that less advanced PD patients very early after diagnosis may respond better to neurotrophic factor gene therapy (Kordower et al., 2013), but the majority of these patients are still fully responsive to L-DOPA substitution therapy and might not accept an irreversible gene transfer as an alternative or adjunct therapy.

We thus asked the question if GDNF gene therapy necessarily needs to be an irreversible process or whether it may be possible to connect therapeutically effective GDNF expression to a controlling drug in a sense that low frequency application of an inducer would allow an intermittent and reversible mode of GDNF expression, with each expression lasting for only relatively short time.

Glial cell line derived neurotrophic factor (GDNF) has demonstrated great success in a number of pre-clinical rodent and non-human primate models of PD. In several toxin-induced lesion paradigms a delayed application of GDNF expressing vectors, i.e. weeks after lesion onset, has had robust beneficial effects on motor behaviour and restoration of dopamine supply in rodents and non-human primates (Drinkut et al., 2012; Eslamboli et al., 2005; Kells et al., 2010; Sajadi et al., 2006). However, GDNF has also demonstrated severe side effects, e.g. substantial weight loss in non-human primates after expression in the substantia nigra (Su et al., 2009). GDNF shows neuroprotective effects already at very low dosage (Eslamboli et al., 2005), but this apparently holds true also for off-target effects, as shown for activation of hypothalamic corticotrophin releasing hormone secretion (Manfredsson et al.,

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2009a). Longer term GDNF expression in the intact nigro-striatal system caused a down-regulation of TH immunoreactivity in dopaminergic nigral neurons over time (Drinkut et al., 2012; Georgievska et al., 2002, 2004) suggesting a potentially problematic influence on neurotransmitter synthesis in surviving or rescued dopaminergic neurons.

To gain advanced control over GDNF expression we used a regulatable AAV vector for GDNF expression that can be induced by short-term application of the inducer mifepristone (Mfp), an approved small molecule drug. This vector allowed for discontinuous GDNF expression, which was demonstrated to be effective in terms of restoration of motor performance and protection of nigral dopaminergic neurons from degeneration in the rat 6-OHDA model of PD.

Our data suggest that it is reasonable and feasible to combine the strengths of pharmacological approaches, i.e. control over application duration and dosage of the therapeutic agent, with the powers of gene therapy, i.e. targeted delivery and long-term persistence of potentially curative transgenes.

Results

Experimental lesion paradigm: slowly progressive 6-OHDA lesion with delayed AAV-GDNF application

We exploited a unilateral, partial 6-OHDA lesion, created by injection of 5 μg 6-OHDA each in two striatal sites. These sites were chosen in the dorso-lateral striatum, where the majority of dopaminergic fibres important for motor control are having their termini. This lesion model has been described to result in a slowly progressive degeneration of the nigro-striatal projection and nigral dopaminergic neurons over a time frame of 3–4 months (Sauer and Oertel, 1994). AAV-5 vectors expressing GDNF either constitutively or from a regulated promoter were injected in animals which demonstrate robust motor impairment at 3 weeks after the lesion (Fig. 1). While the constitutively expressing vector could express GDNF from this time point on, the regulated vectors were induced only another 2 weeks later, i.e. at 5 weeks after the lesion. Thus, the constitutively expressing GDNF vector can be regarded as providing the maximum possible curative effect that can be obtained in this lesion model, allowing us to standardize our data to previous work (Drinkut et al., 2012; Yang et al., 2009).

6-OHDA application resulted in onset of drug induced and voluntary motor phenotypes at 1–2 weeks after toxin injection, which persisted up to 4 months after the lesion (Fig. 3). Striatal dopamine levels were depleted to 40% as compared to un-lesioned controls at 6 weeks after lesion, and this depletion also lasted for 4 months without endogenous recovery (Fig. 4). Numbers of nigral dopaminergic neurons as quantified by both VMAT2 and TH immunoreactive cells were not reduced at 5 weeks after lesion but dropped to about 50% of controls at 4 months after lesion in untreated animals (Fig. 5).

We used of two different induction paradigms to answer the question if a short-term induction of GDNF expression could have sustained therapeutic effects. In the first experiment GDNF expression was induced only once by Mfp application, at two weeks after injecting the recombinant vectors, corresponding to 5 weeks after the 6-OHDA lesion. In a second group we induced GDNF expression for a second time, by Mfp application 5 weeks after the first induction, corresponding to 10 weeks after 6-OHDA lesion. In both cases animals were monitored for up to 17 weeks after 6-OHDA lesion for neurorestorative or protective effects of these treatments.

Regulated GDNF expression

Assessing the kinetics of GDNF induction revealed that at one week after application of mifepristone (Mfp) a striatal tissue level of about 120 pg GDNF/mg tissue was reached, as compared to 5 pg/mg in non-induced and EGFP expressing brains and 260 pg/mg tissue in constitutively GDNF expressing brains (Fig. 2). Two weeks later, i.e. at three weeks after induction, GDNF levels have declined to baseline levels. The second application of Mfp, at two weeks after GDNF levels have dropped to control values, again resulted in 120 pg GDNF/mg tissue at one week after induction. These results indicated that the regulated system reacts with the same kinetics repeatedly. Thus, a short-term application of the inducer Mfp resulted in a robust, but also relatively short-termed expression of GDNF, which has completely returned to baseline at three weeks after this induction. Mfp application or viral vector administration per se had no influence on levels of GDNF as shown for regulated EGFP expressing animals, and no increase over baseline GDNF levels was detected in animals which had been injected with the regulated GDNF vector but did not receive Mfp application (Fig. 2). GDNF levels obtained for all time points investigated and for further control groups of animals are detailed in Supplemental Table 1.

Restoration of motor control

The striatal 6-OHDA lesion as applied in this study resulted in robust onset of apomorphine-induced rotation behaviour at 2 weeks after lesion. Animals that were injected with EGFP expressing control vectors or with the regulated GDNF vectors but did not receive Mfp demonstrated stable rotation behaviour over the course of the study, i.e. up to 17 weeks after 6-OHDA lesion, confirming absence of endogenous recovery from the lesion (Figs. 3a, b).

In contrast, animals that were injected with the constitutively expressing GDNF vector showed a long-term and stable reduction of apomorphine induced rotations by about 80%, indicating that substantial recovery from lesion-induced motor impairment could be achieved if GDNF was constitutively expressed from 3 weeks after the lesion (Fig. 3c).

Rotation behaviour was also substantially reduced by 50–60% in animals expressing the regulated GDNF when induced once with Mfp (Fig. 3d). Of note, in these animals GDNF expression can only start at 5 weeks after the lesion, i.e. two weeks later as compared to the

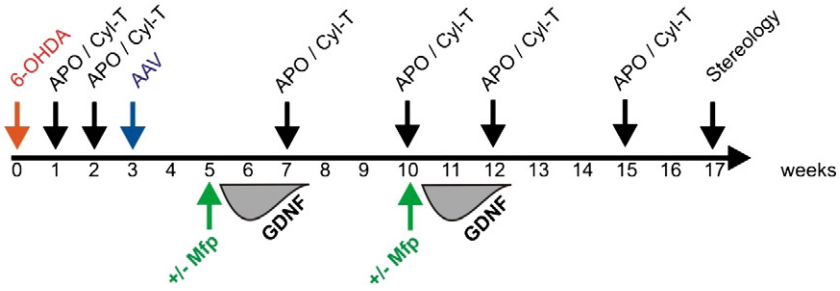

Fig. 1. Time schedule of the study. Rats were subjected to partial striatal 6-OHDA lesion at time point 0 week. Animals were behaviourally examined at 1, 2, 7, 10, 12 and 15 weeks after 6-OHDA by apomorphine induced rotation behaviour (APO) and by the cylinder test for forepaw usage (Cyl-T). Mifepristone in DMSO or DMSO only was applied on three consecutive days either at 5 weeks after 6-OHDA or at 5 and 10 weeks after 6-OHDA (+/− Mfp). Expression of GDNF is schematically shown by grey shaded curves (for details see Fig. 2 and Supplemental Table 1).