Neuroscience 141 (2006) 521–531

CONTINUOUS EXPOSURE TO GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR TO MATURE DOPAMINERGIC TRANSPLANTS IMPAIRS THE GRAFT'S ABILITY TO IMPROVE SPONTANEOUS MOTOR BEHAVIOR IN PARKINSONIAN RATS

C. WINKLER,[a,b] B. GEORGIEVSKA,[c] T. CARLSSON,[a] B. LACAR[a] AND D. KIRIK[a]*

[a]*Department of Experimental Medical Science, Section of Neuroscience, CNS Disease Modeling Unit, Lund University, BMCA11, S-22184 Lund, Sweden*

[b]*Department of Neurology, Hannover Medical School, D-30625 Hannover, Germany*

[c]*Department of Experimental Medical Science, Section of Neuroscience, Neurobiology Unit, Lund University, BMCA11, S-22184 Lund, Sweden*

Abstract—Functional recovery following intrastriatal transplantation of fetal dopaminergic neurons in animal models of Parkinson's disease is, at least in part, dependent on the number of surviving dopaminergic neurons and the degree of graft-derived dopaminergic reinnervation of the host striatum. In the present study, we analyzed whether continuous exposure of glial cell line-derived neurotrophic factor (GDNF) to mature dopaminergic grafts could further boost the functional outcome of widespread intrastriatal dopaminergic grafts. Rats with dopamine-denervating lesions received multiple intrastriatal transplants of fetal dopaminergic cells and graft-induced behavioral effects were analyzed in drug-induced and spontaneous motor behaviors. At three months after grafting, animals received intrastriatal injections of recombinant lentiviral vectors encoding for either human GDNF or the green fluorescent protein. Continuous exposure of GDNF to the grafts did not boost the functional recovery beyond what was observed in the control animals. Rather, in some of the spontaneous motor behaviors, animals in the GDNF-group showed deterioration as compared with control animals, and this negative effect of GDNF was associated with a down-regulation of the tyrosine hydroxylase enzyme. Based on these and our earlier results, we propose that intrastriatal administration of GDNF at the time of or shortly after grafting is highly effective in initially promoting the cell survival and fiber outgrowth from the grafts. However, once the grafts are mature, GDNF's ability to boost dopaminergic neurotransmission follows the same dynamics as for the native nigral dopaminergic neurons, which appears to be dependent on the concentration of GDNF. Since rather low doses of glial cell line-derived neurotrophic factor at nanogram levels appear to saturate these effects, it may be critical to adjust GDNF levels using tightly regulated gene expression systems. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: Parkinson's disease, fetal tissue, transplantation, glial cell line-derived neurotrophic factor, lentiviral vectors, motor behavior.

Intrastriatal transplants of fetal dopamine (DA) neurons can induce long-lasting functional improvements in animal models of Parkinson's disease (PD) and in PD patients (Herman and Abrous, 1994; Lindvall and Hagell, 2000; Winkler et al., 2000, 2005). The magnitude of functional recovery is dependent, at least in part, on the number of surviving DA neurons in the graft and the degree of reinnervation of the host striatum with graft-derived DA fiber terminals. Thus, motor improvements have been more pronounced in PD patients receiving three or more fetuses per putamen as compared with those receiving one to two fetuses only, which was also reflected as higher ^{18}F-DOPA uptake in PET scans (Cochen et al., 2003; Olanow et al., 2003). Similarly, in the rat PD model, transplants containing fewer DA neurons and providing a restricted DA reinnervation of the striatum induce behavioral recovery in drug-induced rotation and simple motor behaviors only, whereas larger grafts which induce a more pronounced striatal reinnervation might also induce functional recovery in complex motor behaviors (Dunnett et al., 1981; Brundin et al., 1988; Mandel et al., 1990; Winkler et al., 2000). Overall, behavioral recovery in the rat PD model seems to be most pronounced when the grafted cells are spread throughout the target structure to induce an even DA reinnervation of the host striatum (Nikkhah et al., 1993; Winkler et al., 1999; Kirik et al., 2001b). However, even in these cases behavioral recovery remains incomplete. While this might be due to several reasons, two possibilities are most likely: First, incomplete recovery might be due to limitations in graft-derived fiber outgrowth into the host brain. Secondly, the host striatum might lack the appropriate support for full function of the grafted neurons.

The maximal graft-derived tyrosine hydroxylase (TH)-positive fiber innervation levels off at 50–60% of the normal density, suggesting that the ability of the denervated striatum to promote further fiber outgrowth from the grafts is lost at this level of innervation density (Kirik et al., 2001b). Previous studies have shown that the DA-denervated striatum exerts growth-stimulating effects on nigral DA neurons (Bjorklund et al., 1983; Doucet et al., 1990;

*Correspondence to: D. Kirik, Wallenberg Neuroscience Center, BMCA11, S-22184 Lund, Sweden. Tel: +46-46-2220564; fax: +46-46-2223436.
E-mail address: Deniz.Kirik@med.lu.se (D. Kirik).

Abbreviations: ANOVA, analysis of variance; AP, anteroposterior; DA, dopamine; DV, dorsoventral; GDNF, glial cell line-derived neurotrophic factor; GFP, green fluorescent protein; ML, mediolateral; PD, Parkinson's disease; rLV, recombinant lentiviral vector; SN, substantia nigra; TH, tyrosine hydroxylase; VM, ventral mesencephalic; VMAT-2, vesicular monoamine transporter 2; 6-OHDA, 6-hydroxydopamine.

0306-4522/06$30.00+0.00 © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2006.03.068

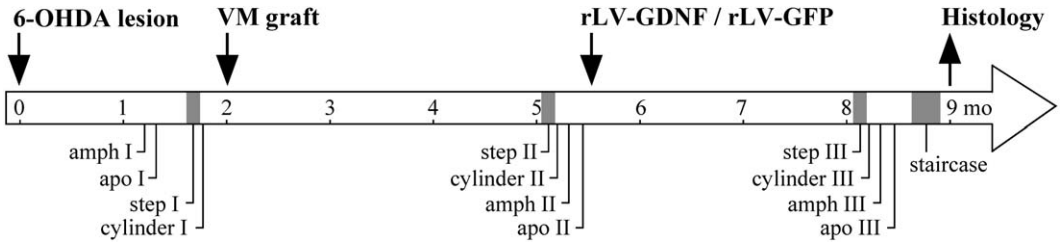

Fig. 1. Time course of surgery and behavioral testing. Performance in rotational behavior induced by either amphetamine or apomorphine and in spontaneous motor behaviors in the stepping and cylinder tests was assessed at three different time-points during the course of the experiment: at 6 weeks after the 6-OHDA lesion and just before transplantation of VM cells; at 3 months after grafting and just before injection of rLV vectors encoding for either GDNF or GFP; and at 14 weeks after rLV-injections prior to killing. During the last test session behavioral performance was also assessed in skilled forelimb use in the staircase test. Animals were perfused at 14 weeks after rLV-injections, i.e. at 7 months after grafting.

Carvey et al., 1996). It is therefore possible that these striatum-derived factors initially stimulate survival and fiber outgrowth from the grafted cells but that they are not sufficient to promote fiber outgrowth above this ceiling value of 50–60% of normal. One of these growth-promoting factors glial cell line-derived neurotrophic factor (GDNF) may be particularly interesting, since it is prominently expressed in the striatum during the first postnatal weeks, i.e. during formation of the terminal DA fields in the striatum, while concentrations are very low during adulthood (Schaar et al., 1993; Strömberg et al., 1993; Choi-Lundberg and Bohn, 1995). Injections or infusions of GDNF into the striatum within a short time interval after transplantation of fetal DA neurons, co-transplantation of GDNF-secreting cells, or viral vector-mediated delivery of GDNF promote the survival of the grafted DA neurons, indicating that GDNF can indeed exert effects on immature DA neurons after transplantation (Rosenblad et al., 1996; Sinclair et al., 1996; Sautter et al., 1998; Wilby et al., 1999; Georgievska et al., 2004a). In addition, enhanced fiber growth from the surviving DA neurons was seen in studies where protein infusion or the encapsulated cells were used. These morphological effects of GDNF were associated with a more pronounced functional recovery as assessed by amphetamine-induced rotation at time-points limited to 4–8 weeks after transplantation.

It is important to note that none of the above studies were aimed at investigating whether grafted DA neurons would function better if they were exposed to GDNF continuously as mature grafted neurons. Thus, in the present study, we analyzed whether long-term expression of GDNF could boost graft-induced functional recovery beyond what is achieved with optimal widespread grafts.

EXPERIMENTAL PROCEDURES

Experimental design

In this study we analyzed the long-term effects of recombinant lentiviral vector (rLV) -mediated overexpression of GDNF on the fiber outgrowth and function of ventral mesencephalic (VM) grafts that were transplanted into the striatum several months prior to injection of viral vectors (Fig. 1). The rats received unilateral injections of 6-hydroxydopamine (6-OHDA) into the striatum in order to induce severe DA-denervating lesions, which are restricted to the striatum and leave the limbic and cortical DA-innervation intact. The lesion-induced behavioral deficits were analyzed in a series of drug-induced and spontaneous motor behaviors and animals were included in the study when they exhibited behavioral deficits that corresponded to a >80% DA-denervation in the striatum. All rats received multiple intrastriatal transplants of fetal VM cells and graft-induced behavioral effects were analyzed in drug-induced and spontaneous motor behaviors. At three months after grafting, animals were divided into two groups (n=9/group) with respect to their scores in the stepping test, and received intrastriatal injections of rLV vectors encoding for either human GDNF or the green fluorescent protein (GFP) in the control group. The functional consequences of these treatments were analyzed in drug-induced and spontaneous motor behaviors before the animals were killed 14 weeks after transduction with rLV vectors. 6-OHDA-lesioned animals that received rLV-GDNF- or rLV-GFP-injections but no transplants, and untreated 6-OHDA-lesioned animals and normal controls have been described in detail in the parallel experiment, in which viral vectors were injected prior to transplantation (Georgievska et al., 2004a). Animals from both studies received injections of the same batch of vectors and the same experimenters did the behavioral testing. The striatal tissue levels of GDNF in this study were estimated to be between 1.6–1.9 ng/mg tissue at 3 and 9 months post-transduction (Georgievska et al., 2004a). This level of GDNF is biologically active since it promotes the survival of intrastriatal DA neuron grafts, when the intrastriatal injection of GDNF-expressing viral vectors is performed prior to transplantation (Georgievska et al., 2004a).

Animals

Thirty female Sprague-Dawley rats (B&K Universal, Stockholm, Sweden) weighing 225–250 g at the beginning of the experiment were used. The animals were housed under a 12-h light/dark cycle with *ad libitum* access to food and water. The housing of the animals and all surgical procedures were performed according to the rules set by the Malmö-Lund Ethical Committee on Animal Research. All efforts were taken to minimize the number of animals used in this study and their suffering.

Lesion and transplantation surgery

All surgical procedures were performed under anesthesia with Hypnorm and Dormicum (Apoteksbolaget, Lund, Sweden) using a stereotaxic frame (Stoelting Co., IL, USA). Injections were performed using a glass capillary with an outer diameter of 60–80 μm attached to a Hamilton syringe. Injection coordinates were calculated in mm with reference to bregma and dura (Paxinos and Watson, 1997). For lesion surgery, rats received injections of 6-OHDA into the right striatum to induce severe DA denervation within the striatum but leaving the limbic and cortical DA system intact (Kirik et al., 1998). Seven micrograms of 6-OHDA (calculated as free base) dissolved in 2 μl of 0.05% ascorbate-saline was injected at four deposits in the striatum at the following