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Research Report

Enhanced GDNF expression in dopaminergic cells of monkeys grafted with carotid body cell aggregates

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ABSTRACT

Striatal carotid body cell aggregates (CBCA) grafts improve parkinsonism in animals and patients with Parkinson's disease. As CB cells contain trophic factors, we investigated the long-term effect of striatal CBCA grafts on nigrostriatal dopaminergic cells in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys receiving unilateral (UL-grafted group, $n=4$) or bilateral (BL-grafted group, $n=3$) CBCA autotransplant. Two MPTP monkeys were sham-operated receiving only Tyrode. For histological analysis, we also included 3 MPTP-untreated and 3 intact animals. Brain [18]F-luorodopa (^{18}F-DOPA)-positron emission tomography (PET) scans were performed to assess dopaminergic function in vivo at baseline, 6 and 12 months after surgery. The number of nigral dopaminergic cells was assessed in UL-grafted animals, and the number of dopaminergic cells expressing glial cell line-derived neurotrophic factor (GDNF) in all groups. After 1 year, animals showed a significant recovery of the parkinsonism (San Sebastian et al., 2007) and PET studies revealed a larger striatal ^{18}F-DOPA uptake in the CBCA-grafted striatum compared to that receiving Tyrode. No differences were found in the number of surviving dopaminergic cells when comparing both subtantia nigra of UL-grafted animals. However, both UL- and BL-grafted animals showed a bilaterally increased number of TH-GDNF immunoreactive nigral neurons compared to intact and MPTP-untreated monkeys, indicating that in addition to the proven long-term motor benefit, CBCA autograft might exert a neuroprotective effect on the surviving dopaminergic cells.

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Abbreviations: 18F-DOPA, [18]F-DOPA; BL-grafted, bilaterally grafted; CB, carotid body; CBCA, carotid body cell aggregates; DA, dopamine; DAergic, dopaminergic; GDNF, glial cell line-derived neurotrophic factor; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PET, positron emission tomography; SNpc, substantia nigra pars compacta; TH-ir, tyrosine hydroxylase-immunoreactive; UL-grafted, unilaterally grafted

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative motor disorder characterized by the selective loss of dopaminergic (DAergic) neurons of the substantia nigra pars compacta (SNpc) and a marked reduction in striatal dopamine (DA) content. Due to the limitations of DAergic drugs in advanced stages of the disease, the usefulness of DA-releasing cells grafts is currently being explored as a therapeutic approach for PD (Cepeda et al., 2007; Freed et al., 2001; Kim et al., 2002; Olanow et al., 2003; Takagi et al., 2005; Watts et al., 2003). Among others, carotid body (CB) is an interesting and suitable DAergic cell source as its glomic cells contain high amounts of DA (Espejo et al., 1998; Hao et al., 2002), express a wide diversity of trophic factors (Belzunegui et al., 2008; Izal-Azcarate et al., 2008; Paciga and Nurse, 2001; Wang and Bisgard, 2005) and hypoxia, which is present within the graft, elicits CB cell growth and DA release (Arias-Stella and Valcarcel, 1976; Bee et al., 1986). Actually, striatal autotransplant of carotid body cell aggregates (CBCA) produces a moderate but long-lasting amelioration of parkinsonism in rats (Espejo et al., 1998; Hao et al., 2002) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Luquin et al., 1999). Furthermore, the first clinical study in PD patients demonstrated a significant and sustained motor improvement, along with a slower decline rate of the striatal [18]F-DOPA (^{18}F-DOPA) uptake from the expected yearly 10% decrement (Hilker et al., 2005) in PD patients receiving striatal CB autografts (Arjona et al., 2003; Minguez-Castellanos et al., 2007).

Although the mechanisms by which CBCA graft ameliorates parkinsonism are uncertain, experimental data suggest that motor recovery is probably due to the release of trophic factors contained in the CB cells (Villadiego et al., 2005). In fact, we have recently demonstrated that CBCA autografts induce long-lasting cellular changes on the population of striatal tyrosine hydroxylase-immunoreactive (TH-ir) cells, probably by the effect of the released glial cell line-derived neurotrophic factor (GDNF) from the glomic cells. This increased number of striatal DAergic cells might have contributed to the sustained motor recovery found in our parkinsonian animals (San Sebastian et al., 2007). In order to further elucidate the mechanisms underlying CBCA graft-induced motor recovery, the striatal DAergic function was assessed in vivo by means of brain ^{18}F-DOPA PET studies undertaken along the survival time of the same CBCA grafted monkeys. In addition, since previous studies indicate that GDNF might exert certain neurotrophic/neuroprotective effects on nigral DAergic cells (Jollivet et al., 2004; Kishima et al., 2004; Palfi et al., 2002), we investigated whether intrastriatal CBCA grafts are able to induce relevant changes in the number and phenotype of nigral DAergic neurons.

2. Results

2.1. Survival of CBCA graft

The graft deposit was identified within the striatum of all grafted animals at the end of the needle scar, near the center of the putamen or mildly displaced in the mediodorsal direction. Systematic cell counting of TH-ir glomic cells before or after grafting was not performed in any of the animals. However, we found a small number of aggregated TH-ir cells within the graft scar with the typical morphology of glomic cells observed (Fig. 1). The total number of surviving glomic cells was roughly estimated to be below 80–100 cells per injection site. The existence of these cells indicates that at least some CB cells survived and still expressed TH, up to 1 year after the graft. We found no TH-ir glomic cells outside the graft limits thus indicating theses cells have not the ability to migrate from the deposit site.

2.2. ^{18}F-DOPA PET outcome

In a previous work, we found that CBCA autograft induced a long-lasting motor amelioration in both unilaterally (UL) and bilaterally (BL)-grafted animals, while sham-operated group did not show any motor recovery along the survival time (San Sebastian et al., 2007). Motor recovery was observed both the global motor assessment and in the performance of fine motor tasks (data no showed). The maximal motor recovery was

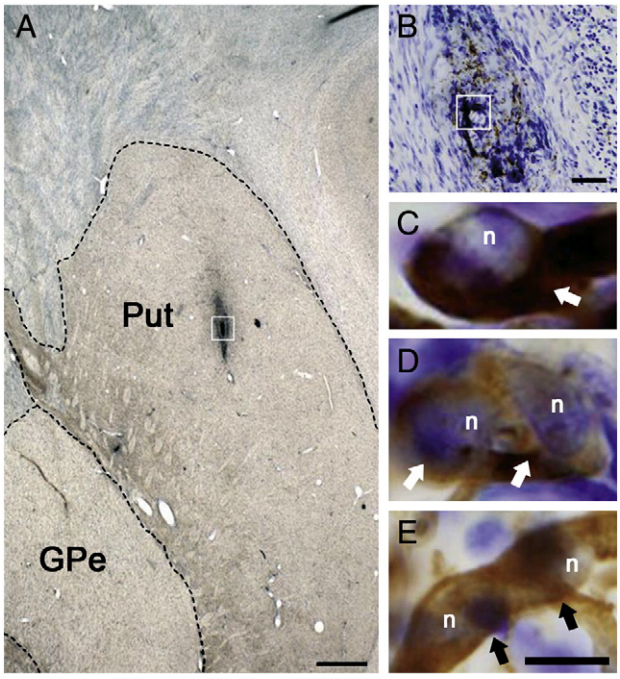

Fig. 1 – Bright-field microscopy images of the graft site in a coronal section of the striatum stained for tyrosine hydroxylase (TH) immunoreactivity and counterstained with Nissl. (A) Photomicrograph of a rostral injection showing CBCA graft in the center of the posterior putamen (white box) and insert (B) where TH-ir structures can be distinguished inside the needle track (white box). At higher magnification, it can be seen that these structures correspond to TH-ir glomic cells inside the same graft (C and D) or in the needle track of a different animal (E). Black and white arrows point to TH-ir cells, where the nucleus (n, Nissl blue) can be seen surrounded by the TH-ir cytoplasm (brown). Put: putamen, GPe: external globus pallidus. TH-ir: tyrosine hydroxylase-immunoreactive Scale bar, 1 mm (A), 50 μm (B) and 10 μm (C–E).