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Research Paper

# Functional Rescue of Dopaminergic Neuron Loss in Parkinson's Disease Mice After Transplantation of Hematopoietic Stem and Progenitor Cells

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ABSTRACT

Parkinson's disease is a common neurodegenerative disorder, which is due to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and for which no definitive cure is currently available. Cellular functions in mouse and human tissues can be restored after fusion of bone marrow (BM)-derived cells with a variety of somatic cells. Here, after transplantation of hematopoietic stem and progenitor cells (HSPCs) in the SNpc of two different mouse models of Parkinson's disease, we significantly ameliorated the dopaminergic neuron loss and function. We show fusion of transplanted HSPCs with neurons and with glial cells in the ventral midbrain of Parkinson's disease mice. Interestingly, the hybrids can undergo reprogramming *in vivo* and survived up to 4 weeks after transplantation, while acquiring features of mature astroglia. These newly generated astroglia produced Wnt1 and were essential for functional rescue of the dopaminergic neurons. Our data suggest that glial-derived hybrids produced upon fusion of transplanted HSPCs in the SNpc can rescue the Parkinson's disease phenotype *via* a niche-mediated effect, and can be exploited as an efficient cell-therapy approach.



## 1. Introduction

Cellular plasticity in mouse and human tissues can be modulated after fusion of bone marrow (BM)-derived cells with a variety of cells, such as neurons, hepatocytes, cardiomyocytes, and gut cells (Nygren et al., 2004; Doyonnas et al., 2004; Ogle et al., 2005; Johansson et al., 2008; de Jong et al., 2012). The hybrids produced can repair the function of these different cell types in a damaged organ (Wang et al., 2003; Johansson et al., 2008; Sanges et al., 2013).

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, and it affects about 1% of the population above the age of 60 years (de Rijk et al., 1997). PD develops due to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with the consequent degeneration of tyrosine-hydroxylase fibers in the striatum (CPu, caudate-putamen), and dopamine (DA) depletion (de Rijk et al., 1997). No definitive cure is currently available for PD.

We aimed here to determine whether transplantation of hematopoietic stem and progenitor cells (HSPCs) in two different PD mouse models can rescue the loss of dopaminergic neurons after fusion. HSPCs are currently used in clinical-trial protocols (Chen et al., 2014a, 2014b; Park et al., 2015), which suggests their safety.

The neurotoxin 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) can induce parkinsonian syndrome in humans that is almost indistinguishable from PD. This treatment produces a dramatic bilateral degeneration of tyrosine-hydroxylase-positive (TH+) neurons in the SN, and of TH+ fibers in the striatum (Bové and Perier, 2012). The neurotoxin 6-hydroxydopamine (6OHDA) triggers death of dopaminergic neurons when injected directly into the brain. Unilateral 6OHDA-injection has also been described as a 'hemiparkinson model', whereby the intact hemisphere serves as the internal control for the resulting asymmetric and quantifiable motor behavior impairment (Bové and Perier, 2012).

Cell therapy of PD is currently pursed in the striatum, through the increase of DA levels (Meyer et al., 2010). Here, however, we transplanted HSPCs into the SNpc of both MPTP and 6OHDA mouse models, with the aim being to regenerate or protect the loss of dopaminergic neurons.

We show that transplanted HSPCs can fuse with neurons and glial cells in both PD models. The glial-derived hybrids survive over the

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long-term after the transplantation, and they acquire features of mature astroglia and secrete Wnt1. A niche-effect was associated with an important attenuation of dopaminergic neuronal degeneration. As a consequence, functional amelioration of the PD phenotype was observed. The activity of the Wnt signaling pathway was required for this phenotype rescue.

## 2. Materials and Methods

### 2.1. Mice

The mice were housed in accordance with the Ethical Committee for Animal Experimentation of the Government of Catalonia, and the experiments were performed in accordance with the rules set by the local Animal Ethics Committee. ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines (Kilkenny et al., 2010) were followed.

All of the mice in this study were generated in a wild-type C57BL/6J background. Here, we used wild-type C57BL/6J and the following transgenic mice: CAG-Cre (Hayashi and McMahon, 2002), β-actin-Cre (Srinivas et al., 2001), CAG-RFP (Long et al., 2005), GFAP-Cre (Gregorian et al., 2009), FoxA2-Cre (Park et al., 2008), R26Y (Srinivas et al., 2001), and ROSA26iDTR (Buch et al., 2005a, 2005b).

### 2.2. Establishment of the MPTP/6OHDA Mouse Models

For the 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) mouse model, 8- to 12-week-old male mice received one intraperitoneal injection of MPTP-HCl per day (30 mg/kg free base; Sigma) for five consecutive days, according to the sub-acute MPTP injection paradigm (Vila et al., 2001). Control mice received 0.9% sterile saline injections only. For the 6-hydroxydopamine (6OHDA) mouse model, either male or female mice had 6OHDA injected into the right substantia nigra (SNpc) pars compacta (Parish et al., 2001), under anesthesia and analgesia (2% isoflurane in 2:1 oxygen/nitrous oxide), using a Kopf stereotaxic frame (Kopf Instruments) and a 5 μl Hamilton syringe fitted with a fine capillary. The 6OHDA was used at 1.5 μg/μl (calculated as the free base; Sigma) dissolved in a solution of 0.2 mg/ml ascorbic acid in 0.9% sterile saline. A single injection of 2 μl was performed using the stereotaxic coordinates according to Paxinos and Franklin (2008): −3.0 mm anterior/posterior, 1.05 mm lateral with respect to the bregma, and −4.7 mm ventral from the dura, with a flat skull position. Injections were made at a rate of 0.25 μl/min with a further 4 min allowed for the toxin to diffuse before slow withdrawal of the capillary, followed by cleaning and suturing of the wound. For all *in vivo* experiments, animals were randomized into cages by the animal facility staff, so that the investigator did not select groups based on size or appearance. Establishment of Parkinson's disease model (MPTP and 6OHDA) and the subsequent treatments received were randomly applied on the animals in each cage. Additionally, experiments with mortality rate over 50% after MPTP intoxication were discarded. The sole exclusion criterion was death of the subject animal (due to complications linked with the surgical procedure).

### 2.3. Cell Preparation and Transplantation

Just before the transplantation, lineage-negative HSPCs were isolated from the total bone marrow of donor mice using Lineage Cell Depletion kits (Miltenyi Biotech). At day 3 after the last MPTP injection or the 6OHDA infusion, the recipient mice were placed into a Kopf stereotaxic frame and received one injection of approximately 60,000 cells suspended in 2 μl phosphate-buffered saline (PBS) over the right substantia nigra (stereotaxic coordinates were determined according to Paxinos and Franklin (2008): −2.9 mm anterior, 1.3 mm lateral with respect to the bregma, and −4.5 mm ventral from the dura, with a flat skull position. The intracerebral injection was performed using a 5 μl Hamilton microsyringe coupled with a 33-gauge needle. Cell infusions were performed at a rate of 0.5 μl/min. On completion of the injection, the needle was left in place for 4 min before being retracted slowly at a rate of 1 mm/min, to avoid reflux along the injection track. After the surgery, the mice were placed under a warm lamp until their complete awakening. To block the Wnt/β-catenin signaling by infusion of Dckkopf-1 (Dkk1) into the SNpc *in vivo*, the recombinant Dkk1 protein (R&D Systems, MN, USA) was dissolved in sterile physiological saline (0.9% NaCl) at a final concentration of 1 μg/μl. One infusion of Dkk1 was carried out unilaterally into the SNpc using a 5 μl Hamilton microsyringe and 2 μg/infusion at the same time of the cell transplantation.

### 2.4. Tamoxifen Treatment for Cre-recombinase Induction and Diphtheria Toxin Injection

To induce Cre recombination for the YFP labeling, 20 mg tamoxifen (T5648, Sigma) was dissolved in 100 μl ethanol and 900 μl corn oil at 56 °C for 2–3 hours. Tamoxifen was stored in the dark at 4 °C until use, for up to 3 weeks. Tamoxifen was warmed to 37 °C before administration. The mice received intraperitoneal injections of a total 75 mg tamoxifen/kg body weight, once per day for three consecutive days. To ablate the hybrid cells, ROSA26iDTR transgenic mice were injected intraperitoneally with 525 ng diphtheria toxin (D0564, Sigma-Aldrich, St. Louis, MO, USA) in 300 ml PBS per injection, for four consecutive days.

### 2.5. Neurochemical Analysis

Tissue DA, DOAPC and HVA content were determined on tissue samples from striatum (CPu, caudate-putamen) and cortex (Ctx) by HPLC with electrochemical detection (Waters model 2465; +0.7 V) as previously described (Bortolozzi and Artigas, 2003). Mice were killed and their brains were quickly removed and placed over a cold plate. Caudate putamen (CPu) and cortex were carefully dissected out, weight, frozen on dry ice and kept at −80 °C until assayed. The tissue were homogenized in 200 μl of buffer containing 0.4 M perchloric acid containing 0.1% sodium metabisulphite, 0.01% EDTA, 0.1% cysteine and centrifuged at 12,000 *g* for 30 min. Aliquots of supernatants were then filtered through 0.45 μm filters (Millex, Barcelona, Spain) and analyzed by HPLC as described. The mobile phase consisted of 0.1 M KH2PO4, 1 mM octyl sodium sulphate, 0.1 mM EDTA (pH 2.65) and 18% methanol. DA and their metabolites were separated on a Mediterranea Sea (C18, 3 μm, 10 cm × 6.4 mm) (Teknokroma, ref TR010042, Barcelona, Spain).

### 2.6. Animal Behavior Studies

The motor responses to sensory stimuli in the MPTP-treated mice were measured using the adhesive removal test. Two training trials were performed prior to the surgery, with an adhesive dot sticker (0.6 cm diameter, Avery) placed on the plantar surface of the forelimb. At 4 weeks after Sham or HSPC transplantation of the MPTP-treated mice, the adhesive dot sticker was placed on the forelimb, and the time to make contact and to remove the sticker from the forelimb was recorded. If a mouse did not remove the sticker within 60 seconds (s), it received a score of 60 s. The mean data were calculated across three trials, at one per day. Immediately after this test, the spontaneous activity test was run in the cylinder, by placing the mice into a small transparent cylinder (height, 15.5 cm; diameter, 12.7 cm). The spontaneous activity was videotaped for 3 minutes, and the number of rears and the time spent grooming were measured. To test forepaw akinesia in the 6OHDA mouse model, lesioned mice were randomly assigned to each treatment group, as the Saline group, the control group (6OHDA + Sham), and the group for the transplantation of the HSPCs. To test the spontaneous use of the forelimbs, the cylinder test was