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Platelet-derived growth factor-BB has neurorestorative effects and modulates the pericyte response in a partial 6-hydroxydopamine lesion mouse model of Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease where the degeneration of the nigrostriatal pathway leads to specific motor deficits. There is an unmet medical need for regenerative treatments that stop or reverse disease progression. Several growth factors have been investigated in clinical trials to restore the dopaminergic nigrostriatal pathway damaged in PD. Platelet-derived growth factor-BB (PDGF-BB), a molecule that recruits pericytes to stabilize microvessels, was recently investigated in a phase-1 clinical trial, showing a dose-dependent increase in dopamine transporter binding in the putamen of PD patients. Interestingly, evidence is accumulating that PD is paralleled by microvascular changes, however, whether PDGF-BB modifies pericytes in PD is not known.

Using a pericyte reporter mouse strain, we investigate the functional and restorative effect of PDGF-BB in a partial 6-hydroxydopamine medial forebrain bundle lesion mouse model of PD, and whether this restorative effect is accompanied by changes in pericyte features.

We demonstrate that a 2-week treatment with PDGF-BB leads to behavioural recovery using several behavioural tests, and partially restores the nigrostriatal pathway. Interestingly, we find that pericytes are activated in the striatum of PD lesioned mice and that these changes are reversed by PDGF-BB treatment.

The modulation of brain pericytes may contribute to the PDGF-BB-induced neurorestorative effects, PDGF-BB allowing for vascular stabilization in PD. Pericytes might be a new cell target of interest for future regenerative therapies.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a progressive loss of the dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and an associated loss of DA striatal fibres (de Rijk et al., 1997). The lack of dopamine in the striatum is associated with typical motor symptoms such as bradykinesia, rigidity, tremor and balance problems (Fearnley and Lees, 1991).

There is an unmet clinical need for effective and long-lasting neurorestorative strategies to prevent further degeneration of nigrostriatal neurons and axons, and to slow disease progression.

Evidence is accumulating that vascular alterations, in particular angiogenesis, may be a possible mechanism involved in the pathogenesis of PD (Faucheux et al., 1999; Janelidze et al., 2015; Wada et al., 2006; Yasuda et al., 2007). Pericytes are one of the key players in angiogenesis and blood-brain-barrier integrity (Armulik et al., 2010). They also contribute to local homeostasis, secrete growth factors (Shimizu et al., 2012) and inflammatory molecules (Kovac et al., 2011) and are multipotent cells (Dore-Duffy, 2008; Ozen et al., 2012; Ozen et al., 2014; Paul et al., 2012). Importantly, cerebral pericytes express platelet-derived growth factor (PDGF) receptor β (PDGFRβ) (Winkler et al., 2010), and are recruited by PDGF-BB-secreting endothelial cells to stabilize newly formed blood vessels (Armulik et al., 2005). PDGF-BB is an endogenous growth factor that not only plays a role in angiogenesis, but also has neuroprotective effects on DA neurons in vitro (Nikkhah et al., 1993; Pietz et al., 1996), and has been shown to restore the nigrostriatal pathway in different partial lesion PD models in vivo (Zachrisson et al., 2011). Based on these findings, PDGF-BB has entered

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clinical trials and safety and tolerability of PDGF-BB have recently been evaluated in PD patients (Paul et al., 2015). However, it is currently unclear whether PDGF-BB can induce long-lasting improvement of symptoms and whether it alters features of pericytes.

Neuroprotective and neurorestorative interventions in PD require models that resemble earlier stages of PD, where some DA cells and fibres still remain. We therefore use a partial lesion PD model in pericyte-reporter mice (Boix et al., 2015). This model allows for a quantifiable behavioural and histological readout in order to study the effect of intracerebroventricular (i.c.v.) administration of PDGF-BB on motor symptoms. 6-hydroxydopamine (6-OHDA) was injected into the medial forebrain bundle (MFB) to avoid injection of the toxin in target regions for neuroprotection and vascular changes, such as the striatum.

We examine if behavioural recovery is associated with nigrostriatal DA pathway restoration in this model immediately and 2 months after treatment. Further, we specifically examine whether a partial 6-OHDA lesion is accompanied by pericyte alterations and whether these changes are affected by PDGF-BB.

2. Material and methods

2.1. Animals

We used 45 male *rgs5*$^{GFP/+}$ knock-out/knock-in mice (C57bl/6 background) (Nisancioglu et al., 2008). In this reporter mouse, the *Green fluorescent protein (GFP)* gene is expressed in the cytoplasm under the pericyte-specific *Regulator of G protein signalling 5* (*RGS5*) promoter, making it possible to track pericytes. Mice have a normal phenotype. Animals were housed in a 12 h/12 h light/dark cycle with access to food and water ad libitum. All experiments were carried out in accordance with the European Directive 2010/63/EU guidelines, and approved by the Ethical Committee at Lund University.

2.2. Partial 6-hydroxydopamine lesion model

A partial MFB 6-OHDA lesion was performed as described previously (Boix et al., 2015). At the day of surgery, animals weighed 25–30 g. Animals were anesthetized with 5% isofluorane (IsoFlo vet, Apoteksbolaget, Sweden), diluted in a 2:1 oxygen:nitrous oxide mixture for 2 min and placed on the stereotaxic frame. Mice received 2% isofluorane throughout the entire surgery (ca. 30 min). 100 μL Marcain (0,25 mg/mL, Apoteksbolaget, Sweden) was injected subcutaneously (s.c.) on top of the skull as a local analgesia, and a skin cut <1 cm was performed to expose the skull. 6-OHDA (Sigma) was dissolved in 0.9% NaCl and 0.02% ascorbic acid to reach a final concentration of 1 μg/μL, and the solution was used within the next 3 h. 6-OHDA was injected into the right MFB at the following coordinates from the bregma: antero-posterior (A/P) = −1.2; medio-lateral (M/L) = −1.3; and dorso-ventral (D/V) = −4.75 (according to the dura mater), using a 10 μL-Hamilton syringe connected to a glass capillary (tip diameter ca. 50 μm), with a flow rate of 0.5 μL/min (final volume = 1 μL). Sham-lesioned animals received only 1 μL of 0.9% NaCl with 0.02% ascorbic acid. Sham-lesioned animals are referred to as controls hereafter. The glass capillary was left in place for the following 5 min to avoid backflow, before being slowly retracted. The skin was sutured, animals received 0.9% NaCl s.c. (1 mL) and daily care the first week with food soaked in 10% sucrose and high calorie jelly food (DietGel Boost, Clear H20 Co.). Animal weight loss was monitored not to be >15% of the initial weight.

2.3. Behavioural tests

Three weeks after the 6-OHDA lesion, animals underwent a battery of behavioural tests to assess the degree of the unilateral nigrostriatal lesion. Mice with a partial lesion ($n = 38$ out of $n = 45$) were selected as previously described (Boix et al., 2015). Briefly, we have previously shown that certain behavioural tests can reliably predict the degree of cell loss in MFB lesioned mice, whereby the stepping test and the amphetamine-induced rotation test are the best predictors (for details see Boix et al., 2015). Four animals had a total lesion, one only a mild lesion and two animals died before the end of the experiment.

For all tests, animals were habituated to the room at least 2 h before the test. The same behavioural tests were then performed 13–15 weeks after the 6-OHDA lesion (Fig. 1).

2.3.1. Corridor test

For body lateralization assessment, animals performed the corridor test, adapted for mice (Grealish et al., 2010). Briefly, animals were food-restricted and kept at 85% of their initial body weight for the entire test time (7 days). They were first habituated to the corridor (60 cm-long, 4 cm-wide and 15 cm-high) with sugar pellets randomly scattered on the floor, 10 min per day, for the first 2 days. From day 3 to day 7, animals were habituated to an empty corridor for 5 min, and then placed in a corridor presenting 10 pots on each side, spaced by 5 cm and containing 2 to 3 sugar pellets in each. Contralateral and ipsilateral retrievals were scored until either 20 retrievals were counted or 5 min elapsed.

2.3.2. Stepping test

To assess forelimb akinesia, the stepping test was used as previously described for mice (Blume et al., 2009). Mice were lifted gently from their tail and pulled backward in a 50 cm-long, 10 cm-wide corridor, within 10 s. The test was repeated 3 to 5 times per animal, and the whole experiment was videotaped and analysed using a slow motion video player (VLC software). Contralateral and ipsilateral forelimb adjusting steps were quantified.

2.3.3. Cylinder test

Mice performed the cylinder test to evaluate spontaneous forelimb lateralization (Schallert et al., 2000). Animals were placed in a glass cylinder (19 cm-diameter, 20 cm-high) with mirrors behind to allow a 360° vision. Mice were left to explore for 3 min, and the whole experiment was videotaped and analysed using a slow motion video player (VLC software). Contralateral and ipsilateral wall touches (contact with full digits) were quantified.

2.3.4. Drug-induced rotation tests

To assess rotational asymmetry, animals were tested with apomorphine and amphetamine (Ungerstedt and Arbuthnott, 1970). Mice were placed in an automated rotometer apparatus (Omnitech electronics) consisting of a glass bowl (50 cm-diameter) covered with bedding, where mice were harnessed and left to habituate for 15 min. For apomorphine-induced rotation test, animals were primed 4 and 2 days before the test with a s.c. injection of apomorphine (Sigma) at a concentration of 0.1 mg/kg, dissolved in 0.9% NaCl and 0.02% ascorbic acid. They received the same dose on the test day. Contralateral and ipsilateral turns were recorded for 40 min, and the net contralateral turns were calculated. At least 3 days after, animals were placed in the

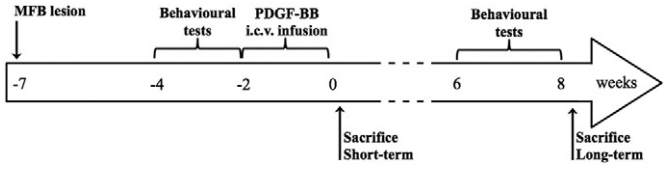

Fig. 1. *Design of the experiment.* Animals were lesioned in the MFB with 6-OHDA or saline (controls) and allowed to recover for the next 3 weeks. Mice then underwent a battery of behavioural tests to select partially lesioned mice, before receiving either PDGF-BB or vehicle treatment into the lateral ventricle for 2 weeks. Once the mini-pump was removed, animals were either sacrificed immediately after treatment, or kept for 6 more weeks, where behavioural tests were performed 6 to 8 weeks after the treatment, before mice were sacrificed. MFB: medial forebrain bundle; i.c.v.: intracerebroventricular.