Brain, Behavior, and Immunity 61 (2017) 155–164

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Full-length Article

Influence of chronic L-DOPA treatment on immune response following allogeneic and xenogeneic graft in a rat model of Parkinson's disease

Ludivine S. Breger [a,b,*], Korbinian Kienle [a], Gaynor A. Smith [b], Stephen B. Dunnett [b], Emma L. Lane [a]

[a] School of Pharmacy & Pharmaceutical Sciences, Redwood Building, King Edward VII Avenue, CF10 3NB Cardiff, UK
[b] Brain Repair Group, Cardiff School of Biosciences, Museum Avenue, CF10 3AX Cardiff, UK

ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 7 November 2016
Accepted 14 November 2016
Available online 15 November 2016

Keywords:
Parkinson's disease
L-DOPA
Transplant
Stem cell
Dyskinesia
Immune response

ABSTRACT

Although intrastriatal transplantation of fetal cells for the treatment of Parkinson's disease had shown encouraging results in initial open-label clinical trials, subsequent double-blind studies reported more debatable outcomes. These studies highlighted the need for greater preclinical analysis of the parameters that may influence the success of cell therapy. While much of this has focused on the cells and location of the transplants, few have attempted to replicate potentially critical patient centered factors. Of particular relevance is that patients will be under continued L-DOPA treatment prior to and following transplantation, and that typically the grafts will not be immunologically compatible with the host. The aim of this study was therefore to determine the effect of chronic L-DOPA administered during different phases of the transplantation process on the survival and function of grafts with differing degrees of immunological compatibility. To that end, unilaterally 6-OHDA lesioned rats received sham surgery, allogeneic or xenogeneic transplants, while being treated with L-DOPA before and/or after transplantation. Irrespective of the L-DOPA treatment, dopaminergic grafts improved function and reduced the onset of L-DOPA induced dyskinesia. Importantly, although L-DOPA administered post transplantation was found to have no detrimental effect on graft survival, it did significantly promote the immune response around xenogeneic transplants, despite the administration of immunosuppressive treatment (cyclosporine). This study is the first to systematically examine the effect of L-DOPA on graft tolerance, which is dependent on the donor-host compatibility. These findings emphasize the importance of using animal models that adequately represent the patient paradigm.

1. Introduction

There has been a longstanding debate regarding the potential toxicity of the mainstay therapy for the neurodegenerative movement disorder Parkinson's disease (PD), L-DOPA. It has been hypothesized that the drug may impact on the development of the disease by hastening or preventing nigral degeneration in PD patients (Quinn et al., 1986; Diamond et al., 1987; Rajput et al., 1997; Fahn et al., 2004). While studies suggest it is not clinically relevant to disease progression, *in vitro* studies have demonstrated that dopaminergic neurons in culture are vulnerable to the oxidative damage caused by L-DOPA (reviewed by Olanow (2015)). The possibility of toxicity however becomes particularly relevant when looking at curative or cell replacement strategies for the treatment of PD.

Fetal cell transplantation of dopaminergic neurons into the caudate putamen was first trialed in 1989 (Lindvall et al., 1990b). Having shown encouraging results in preclinical studies and open-label clinical trials, US-led double-blind placebo controlled studies failed to demonstrate consistent benefit from the graft (Lindvall et al., 1990a; Kordower et al., 1995; Hauser et al., 1999; Hagell and Brundin, 2001). Furthermore these studies, alongside the retrospective video analysis of the London-Lund-Marburg open label study, blew the field into disarray with the discovery of motor side effects persisting after the withdrawal of L-DOPA, now termed graft-induced dyskinesias (GID) (Hagell et al., 2002; Olanow, 2003). In the search to understand inconsistency in transplant efficacy and the source of the motor side effects, it is important to consider factors that are present in patients but absent in models of transplantation in PD (generally the 6-hydroxydopamine (6-OHDA) lesioned rat). In this context, L-DOPA toxicity may be of greater relevance as, at the early stage

* Corresponding author at: Dept of Experimental Medical Science, Wallenberg Neuroscience Centre, Lund University, BMC A11, 221 84 Lund, Sweden.
E-mail addresses: Lu.Breger@icloud.com, Ludivine.breger@med.lu.se (L.S. Breger), kienle@ie-freiburg.mpg.de (K. Kienle), Gaynor.Smith@umassmed.edu (G.A. Smith), Dunnettsb@cardiff.ac.uk (S.B. Dunnett), LaneEL@cardiff.se.uk (E.L. Lane).

http://dx.doi.org/10.1016/j.bbi.2016.11.014
0889-1591/

when they are transplanted, developing neurons may be vulnerable to the effects of pulsatile dopamine flux. The majority of transplant recipients will have been on L-DOPA medication for some time prior to transplantation and will remain on it for a significant period post transplantation, as the graft matures enough to support effective dopamine function.

Preclinical studies have reported contradictory findings: some researchers have described failure of the graft to thrive under L-DOPA treatment (Yurek et al., 1991; Steece-Collier et al., 2009) while others found no detrimental effect of the treatment on the survival of grafted dopaminergic cells or their functional efficacy (Blunt et al., 1990, 1991, 1992; Adams et al., 1994). Nonetheless, the role of L-DOPA administration pre- and post-transplantation has not been investigated experimentally in a systematic manner. Furthermore, most of these papers have used ventral mesencephalon (VM) harvested from the same strain of rat as the hosts, in order to avoid a graft-induced immune response. While simplifying the model, this has again neglected a factor, which is critical when considering the transplantation of patients. To the best of our knowledge, only one paper has thus far combined non-syngeneic VM transplants and L-DOPA treatment (Soderstrom et al., 2008). In that paper, L-DOPA was administered to all groups with the focus being to explore the impact of inflammation on the synaptic reorganization occurring the presence of L-DOPA. The study was however not designed to compare the impact of L-DOPA treatment pre- and post- transplantation in a systematic manner.

In determining the effect of L-DOPA on transplanted fetal dopaminergic precursors it is therefore paramount to apply this technique in an improved simulation of 'real world' conditions. The use of syngeneic tissue does not trigger a significant immune response in rodents. Patients receive pooled allogeneic tissue from several donors and post-mortem analysis performed on transplanted patients has illustrated that, even in well surviving grafts, there are infiltrating B- and T-lymphocytes in the grafted putamen indicative of an inflammatory host response in the graft (Kordower et al., 1997). Human genetic diversity is such that, an allograft paradigm is insufficiently aggressive to model the immunogenicity that would be associated with transplanting pooled tissue coming from multiple donors, as is the case in fetal transplantation for Parkinson's disease. Consequently using a donor from a closely related species (e.g. mouse into rat), termed a concordant xenograft, reproduces many aspects of the humoral response observed in transplants for MHC-mismatched allotransplants and would be a more appropriate animal model (Sanchez-Mazas, 2007). This idea has already been used in primates in whom concordant xenografts are used to model human allotransplantation (Michler et al., 1996). This provides a more representative model of the pooled unmatched allograft used to treat PD, than those models used previously. The present study was therefore designed to specifically address the hypothesis that L-DOPA may impact upon graft survival and function in an immunologically incompatible graft. This paradigm is as akin to transplantation conditions in patients as possible, assessing the survival and function of allogeneic and xenogeneic VM grafts while subject to different L-DOPA administration regimes.

2. Materials and methods

2.1. Experimental design

122 female Sprague Dawley rats were unilaterally lesioned using 6-OHDA HBr and split into balanced experimental groups based on the severity of motor impairment following lesion as evaluated by amphetamine-induced rotations, cylinder, vibrissae and stepping tests. The animals were chronically treated with saline or L-DOPA for 8 weeks (phase I: daily for 4 weeks then every other day) before receiving either: an allograft, a xenograft or sham surgery. Animals receiving xenotransplants were treated daily with cyclosporine A (CSA) to avoid rapid rejection of the graft and better mimic the human conditions. In contrast, allografted animals did not receive any immunosuppressive treatment, as allografts are usually well tolerated in rats. Moreover, this would allow a full immunological response to be observed, should the presence of L-DOPA have an impact. All animals were treated again either with L-DOPA or saline for another 8 weeks following grafting (Fig. 1, phase II: daily for 4 weeks then every other day). Dyskinesias were assessed once a week during the treatment phases I and II, both 'on' and 'off' L-DOPA (Scale B, as described in Breger and colleagues (2013)). After 2 weeks to allow complete washout of the drug, motor test were repeated. At the end of the experiment all animals were transcardially perfused with 1.5% paraformaldehyde (Torres et al., 2006).

To verify that there was no overt behavioral consequence of, or interaction between chronic CSA treatment on L-DOPA-induced dyskinesia, a supplementary experiment was carried out. 16 female Sprague Dawley received unilateral 6-OHDA lesions and were selected based on the results of the amphetamine-induced rotation score. They were then separated in 2 groups: 1) treated daily with L-DOPA and CSA (n = 8), 2) treated daily with L-DOPA only (n = 8). Abnormal involuntary movements (AIMs) were scored twice a week, for 4 weeks (L-DOPA 6 mg/kg) and then once a week, for 2 weeks (L-DOPA 12 mg/kg), rotational behavior of the rats was recorded concomitantly.

2.2. Animals and materials

Sprague Dawley rats (experiment 1: n = 122 and experiment 2: n = 16; Harlan, UK) weighing 200–220 g at the start of the experiment were housed 2–4 per cages with *ad libitum* access to food and water. The experiments were approved by the Cardiff University AWERB and carried out in accordance with the UK guidelines for the care and use of experimental animals under Home Office License No 30/3036 and European Communities Council Directive (2010/63/EEC). 6-OHDA, L-DOPA and benserazide were obtained from Sigma Aldrich, UK; cyclosporine (250 mg/5 ml) was obtained from Sandoz Pharmaceuticals, UK. Four animals were excluded from the first experiment due to poor heath, unrelated to the experimental treatments.

2.3. Surgery

2.3.1. 6-Hydroxydopamine lesion

6-OHDA was used to create hemi Parkinsonism in rats. The toxin was delivered directly into the right medial forebrain bundle (MFB), which contains the dopaminergic nigrostriatal pathway. Selective targeting of this site allows near total depletion of nigrostriatal dopaminergic neurons as described by Torres et al. (2011). Briefly, the rats were anaesthetized with 2–3% Isoflurane (IVAK, UK) in a 2:1 $O2/NO_2$ mix and received an infusion of 3 µl of a 30 mM solution of 6-OHDA.HBr (Sigma, UK) containing 0.03% acid ascorbic into the right nigrostriatal pathway at the following coordinates AP −4.0 mm from bregma; ML +1.3 mm from midline; DV −7.0 mm below dura, with the nose-bar set at −4.5 mm below the interaural line. The cannula remained in place for 3 min following injection. Postoperatively, animals were sutured and received a 5 ml injection s.c. of 0.9% sodium chloride containing 4% glucose for hydration and 10 µl of analgesic (5 mg/ml Meloxicam, Boehringer Ingleheim, Germany). Post-mortem analysis confirmed that all animals had greater than 95% loss of tyrosine hydroxylase (TH) positive cells in the right substantia nigra (no significant difference