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Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease

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Abstract

The present study investigated the ability for adult rat neural progenitor cells to survive transplantation, structurally repopulate the striatum and improve motor function in the quinolinic acid (QA) lesion rat model of Huntington's disease. Neural progenitor cells were isolated from the subventricular zone of adult Wistar rats, propagated in culture and labeled with BrdU (50 μM). Fourteen days following QA lesioning, one group of rats ($n = 12$) received a unilateral injection of adult neural progenitor cells (~180,000 cells total) in the lesioned striatum, while a second group of rats ($n = 10$) received a unilateral injection of vehicle only (sham transplant). At the time of transplantation adult neural progenitor cells were phenotypically immature, as demonstrated by SOX2 immunocytochemistry. Eight weeks following transplantation, ~12% of BrdU-labeled cells had survived and migrated extensively throughout the lesioned striatum. Double-label immunocytochemical analysis demonstrated that transplanted BrdU-labeled progenitor cells differentiated into either astrocytes, as visualized by GFAP immunocytochemistry, or mature neurons, demonstrated with NeuN. A proportion of BrdU-labeled cells also expressed DARPP-32 and GAD_{67}, specific markers for striatal medium spiny projection neurons and interneurons. Rats transplanted with adult neural progenitor cells also demonstrated a significant reduction in motor function impairment as determined by apomorphine-induced rotational asymmetry and spontaneous exploratory forelimb use when compared to sham transplanted animals. These results demonstrate that adult neural progenitor cells survive transplantation, undergo neuronal differentiation with a proportion of newly generated cells expressing markers characteristic of striatal neurons and reduce functional impairment in the QA lesion model of Huntington's disease.

Keywords: Huntington's disease; Quinolinic acid lesion model; Adult neural progenitor cells; Subventricular zone; Adult brain; Transplantation

Introduction

Huntington's disease is an autosomal dominant genetic disorder caused by an expansion mutation of a naturally occurring trinucleotide (CAG) repeat in exon 1 of the IT15 gene, encoding a 350-kDa protein termed huntingtin (Huntington's Disease Collaborative Research Group, 1993). This mutation results in excessive involuntary movements (chorea) accompanied by cognitive deficits and behavioural changes due to the progressive and selective degeneration of striatal GABAergic medium spiny projection neurons. At present, there is no clinical treatment to prevent or reduce the onset or progression of Huntington's disease. Cell transplantation therapy may offer a viable treatment strategy for patients with Huntington's disease. Indeed, there is much evidence from animal studies showing that neuronal replacement and partial reconstruction of neuronal circuitry is possible following cell transplantation (Lindvall, 1995; Gage, 1998; Bjorklund and Lindvall, 2000; Nakao and Itakura, 2000). In particular, cell transplantation therapy appears to be well justified for patients with Huntington's disease and previous studies have demonstrated the functional efficacy of transplanting developing striatal neurons in several rodent and primate models of Huntington's disease (Wictorin, 1992; Bjorklund et al., 1994; Dunnett, 1995; Kendall et al., 1998; Palfi et al., 1998). This has lead to the commencement of clinical trials in several centers for cell transplantation therapy for Huntington's disease using intrastriatal implantation of human fetal striatal tissue (Philpott et al., 1997; Kopyov et al., 1998; Rosser and Dunnett, 2003).

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However, the use of human fetal striatal tissue for transplantation therapy in Huntington's disease is associated with major problems: the scarcity of this material is compounded by practical issues such as the age of the donor, viability, contamination and heterogeneity of the tissue as well as major overwhelming ethical and moral concerns (Bjorklund, 1993). The use of a renewable and expandable neural progenitor cell population would circumvent many of the practical and ethical problems associated with the use of freshly harvested human fetal tissue.

The expansion of neural progenitor cells in vitro for transplantation therapy holds several advantages over the use of primary fetal tissue. In vitro expansion has the potential to provide an unlimited, renewable source of donor cells, with the potential to pre-differentiate or regulate gene expression to enhance the differentiation and survival of the transplanted cells. Among the potential candidates for neural transplantation are adult neural progenitor cells, which have been identified in distinct regions of the adult central nervous system (CNS) including the hippocampus, subventricular zone (SVZ), septum, striatum, cortex, optic nerve and spinal cord (Lois and Alvarez-Buylla, 1993; Palmer et al., 1995; Weiss et al., 1996; Shihabuddin et al., 1997; Palmer et al., 1999). In the hippocampus, new neurons are produced from progenitor cells residing in the subgranular zone (SGZ) of the dentate gyrus (Altman and Das, 1965; Kaplan and Hinds, 1977; Eriksson et al., 1998; Gould et al., 1999; Kornack and Rakic, 1999). Similarly, progenitor cells persist and continue to proliferate in the SVZ lining the lateral ventricles (Altman, 1969; Kaplan and Hinds, 1977; Lois and Alvarez-Buylla, 1994; Lois et al., 1996; Thomas et al., 1996). However, unlike the dentate gyrus, SVZ neuronal precursor cells migrate long distances via the rostral migratory stream (RMS) to their final destination in the olfactory bulb where they differentiate into granule and periglomular neurons (Luskin, 1993; Lois and Alvarez-Buylla, 1994; Lois et al., 1996; Thomas et al., 1996). Neural progenitor cells can be isolated from either the SGZ or the SVZ in the adult brain and grown in culture in response to basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) as clonal neurospheres, consisting of neural stem, progenitor and differentiated cells (Reynolds and Weiss, 1992; Gobbel et al., 2003). While these cells express a more restricted fate than embryonic stem cells, they have the potential to give rise to all of the major cells types of the CNS and may provide an alternative cell source for transplantation therapy. Indeed, the development of in vitro methods for expanding adult neural progenitor cells may eventually allow for autologous transplantation.

Adult neural progenitor cells have been transplanted into both neurogenic and non-neurogenic regions of the adult brain with various degrees of success. The majority of studies demonstrated that transplantation of either adult hippocampal-derived or SVZ-derived neural progenitor cells into homotrophic or heterotrophic neurogenic sites results in site-specific neuronal differentiation (Gage et al., 1995; Suhonen et al., 1996; Herrera et al., 1999; Richardson et al., 2005b). In contrast, transplantation into non-neurogenic sites such as the striatum or cerebellum results in the majority of transplanted cells differentiating into glia (Suhonen et al., 1996; Herrera et al., 1999; Dziewczapolski et al., 2003; Richardson et al., 2005a). These studies indicate that regional environmental cues present in the adult brain play a strong role in determining the lineage potential of transplanted adult neural progenitor cells.

It is therefore important to consider how environmental changes after a specific CNS lesion can affect the differentiation potential of transplanted adult neural progenitor cells. Recent studies have demonstrated that transplantation of adult neural progenitor cells into the dopamine-depleted adult rat striatum does not result in significant neuronal differentiation (Dziewczapolski et al., 2003; Richardson et al., 2005a), suggesting that dopaminergic nigrostriatal degeneration does not produce the appropriate signals to induce neuronal differentiation from transplanted adult neural progenitor cells. In contrast, we recently demonstrated progenitor cell proliferation and neurogenesis in the subependymal layer of the caudate nucleus of the basal ganglia in the Huntington's diseased human brain (Curtis et al., 2003). Furthermore, we also demonstrated that the selective loss of GABAergic medium spiny projection neurons in the quinolinic acid (QA) lesion rat model of Huntington's disease results in increased progenitor cell proliferation and the generation of new neurons in the QA lesioned striatum (Tattersfield et al., 2004). This suggests that the correct environmental cues exist in the Huntington's disease brain to direct adult neural progenitor cells to form new mature neurons.

Adult neural progenitor cells may therefore provide an alternative cell source for transplantation therapy for Huntington's disease. In this study, we examined the ability of adult neural progenitor cells isolated from the SVZ of the rat brain to survive transplantation, undergo neuronal differentiation and improve motor function impairment in the QA lesion rat model of Huntington's disease. Fourteen days following QA lesioning of the striatum, rats were transplanted with adult neural progenitor cells that had been expanded in culture and labeled with bromodeoxyuridine (BrdU) prior to transplantation. Adult neural progenitor cells survived transplantation into the lesioned striatum and differentiated into both neurons and astrocytes. A proportion of newly generated cells expressing the neuronal marker NeuN also co-expressed DARPP-32 and GAD_{67}, specific markers for striatal medium spiny projection neurons and interneurons. Moreover, transplantation of adult neural progenitor cells significantly reduced motor dysfunction observed following striatal QA lesioning. Overall, these results demonstrate that adult neural progenitor cells survive transplantation, undergo neuronal differentiation with a proportion of newly generated cells expressing markers characteristic of striatal neurons and reduce functional impairment in the QA lesion rat model of Huntington's disease.

Materials and methods

Animals

Adult male Wistar rats weighing 250–300 g (University of Auckland Animal Resources Unit) were used in this study. The