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Research article

Survival of transplanted human neural stem cell line (ReNcell VM) into the rat brain with and without immunosuppression

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SUMMARY

Functional replacement of specific neuronal populations through transplantation of neural tissue represents an attractive therapeutic strategy for treating neurodegenerative disorders like Parkinson's disease (PD). Even though the brain is a partially immune privileged site, immunosuppression is still needed for the prevention of host immune response, and thus, xenograft rejection. Here, we investigated the fate of human ventral mesencephalon derived immortalized cell line ReNcell VM upon unilateral transplantation into the intact rat striatum with or without immunosuppression with cyclosporine A (CsA).

The status of xenografted human ReNcell VM cells was analysed by immunohistochemistry/immunofluorescence 4 and 6 weeks after transplantation.

Four weeks after transplantation, ReNcell VM cells could be detected in both groups, although the number of survived cells was significantly higher in brains of immunosuppressed rats. In contrast, only 2 out of 6 brains grafted without immunosuppression revealed human ReNcell VM cells 6 weeks post grafting, whereas a considerable number of human cells could still be found in all the brains of immunosuppressed rats. Immunohistochemical analysis of grafted cells showed almost no evidence of neuronal differentiation, but rather astroglial development.

In summary, we have shown that the immunosuppression is needed for the survival of human VM derived progenitor cells in the rat striatum. CsA affected cell survival, but not differentiation capacity: in both groups, grafted either with or without immunosuppression, the ReNcell VM cells lacked neuronal phenotype and developed preferentially into astroglia.

1. Introduction

Neurodegenerative diseases are an assortment of central nervous system (CNS) disorders, characterized by neuronal loss in various brain areas.

Although neurodegenerative diseases have different causes, the dysfunction and loss of certain groups of neurons is common to all these disorders and allows the development of similar therapeutic strategies for their treatment.

There is a great hope for the transplantation of neural progenitor cells (NPCs) in the treatment of progressive neurodegenerative diseases like Parkinson's disease (PD) or Huntington's disease (HD) (Dunnett et al., 1997; Lindvall and Björklund, 2004; Lindvall et al., 2004).

Parkinson's disease (PD) is the second most-common neurodegenerative disease of genetic, toxic as well as slow progressing idiopathic aetiology affecting around 2% of the population over 65 years of age (Roybon et al., 2004). The pathological hallmarks include progressive loss of dopaminergic (DAergic) projection neurons in the substantia nigra (SN) pars compacta and cytoplasmatic inclusions called Lewy bodies (Dawson and Dawson, 2003). The depletion of dopamine (DA) transmitter in the striatum is clinically associated most often with tremor, rigidity, progressive bradykinesia and postural instability (Tedroff, 1999). Idiopathic PD symptoms become apparent when about 50% of nigral DAergic neurons and 70–80% of striatal dopamine are lost (Dunnett and Björklund, 1999). Pharmacological replacement of DA with its precursor L-DOPA (L-3,4-dioxyphenylalanine) works initially, but is often related to undesirable side effects, including motor complications (Fahn et al., 2004).

An effective means to restore motor impairment in animal models of PD, and, potentially, in PD patients, is the replacement of degenerated DAergic neurons through foetal mesencephalic cells (Piccini et al., 2000; Winkler et al., 2005; Mendez et al., 2008).

The capacity of neural progenitor cells to proliferate in cell culture and their ability to differentiate into multiple neuronal cell

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types has made them a powerful tool for experimental studies. The neural progenitor cells derived from different species have been shown to survive when transplanted into animal models of PD, integrate in the host brain and express neuroprotective factors, thus providing behavioural improvement (Blandini et al., 2010; García et al., 2011; Jungnickel et al., 2011; Michel-Monigadon et al., 2011; Ratzka et al., 2011). However, many issues have to be resolved before neural transplantation can be routinely used as a therapeutic approach for Parkinson patients. One of the most crucial issues is the requirement of toxic immunosuppressive drugs.

Even though the brain is an organ with a partial immune privilege, the grafting of tissue from a relative distant species is often associated with different immunological problems, which arise to graft rejection and the greater the phylogenetic distance between donor and host, the more rapid this rejection process.

One of the currently used immunosuppressive drugs in the neuronal transplantation is cyclosporine A (CsA). CsA is a lipophilic cyclic polypeptide that produces calcium-dependent, specific, reversible inhibition of transcription of proinflammatory cytokines (Kaminska et al., 2004). CsA therapy is, however, limited by its side-effects with the most important being: nephrotoxicity, hypertension, hyperlipidemia, changes in mental state and neurotoxicity (Bartynski et al., 2001). In animal studies the application of CsA is limited by long-term costs, contribution to the therapeutic benefit and difficulties in assuring repeated behavioural testing over long survival times. Low doses of CsA have been demonstrated to protect brain from 6-OHDA neurotoxicity (Borlongan et al., 2002). CsA has been suggested to exert neuroprotective effects through inhibition of the mitochondrial permeability transition pore, thus preventing mitochondrial dysfunction in acute and chronic neurodegenerative diseases, insults and global ischemia (Keep et al., 2001; Friberg and Wieloch, 2002; Sinigaglia-Coimbra et al., 2002).

So far, the effectiveness of CsA treatment in xenotransplantation is debatable; a study, that directly compared the percentage of surviving porcine grafts between untreated and CsA treated rat recipients, showed no difference in survival rate between groups at any of time points compared (2, 6, 12 weeks) (Larsson and Widner, 2000). The same group reported later that CsA has a favourable effect on graft survival when combined with pre-treatment of grafted tissue with an inhibitor of lipid peroxidation (Wennberg et al., 2001).

In the present study we compared the survival and differentiation patterns of foetal ventral mesencephalon (VM) derived immortalized cell line ReNcell VM after xenotransplantation with and without immunosuppression.

ReNcell VM is a stable cell line, which is commercially available for research purposes. The cell line has been investigated by our group in *in vitro* studies, using immunocytochemistry, Western blot and proteome analysis (Hoffrogge et al., 2006; Lange et al., 2011). The cells have been shown to display properties of a stem cell, expressing nestin in undifferentiated state and undergoing differentiation in neuronal and glial direction after refraction of growth factors from cell culture medium.

2. Materials and methods

2.1. Cell culture

The transplantation experiments were performed with a stable human foetal midbrain stem cell line, ReNcell VM (ReNeuron, Ltd., Guildford, UK). The cell line was derived from 10-week-old gestational ventral mesencephalon brain tissue and was immortalized with the v-myc oncogene. ReNcell VM were cultivated essentially as described elsewhere (Hoffrogge et al., 2006; Miljan et al., 2009).

2.2. Animals

A total of 25 male Wistar rats weighing between 100 and 200 g at the beginning of the experiment were housed at 22 ± 2 °C under 12-h light/dark cycle with free access to food and water. All animal-related procedures were conducted in accordance with local ethical guidelines and approved animal care protocols.

2.3. Immunosuppression

Cyclosporine A (Sandimmune®, Novartis) was daily injected intraperitoneally for the duration of the experiment, beginning 36 h before transplantation. Cyclosporine A was injected at a dosage of 10 mg/kg. Antibacterial prophylaxis was administered by addition of Borgal (40 mg trimethoprim, 200 mg sulphadoxine per ml) to the drinking water. Control animals were treated instead of CsA with 0.9% sodium chloride.

2.4. Intrastriatal cell transplantation

All surgery was performed under full anaesthesia by a mixture of ketamine and xylazine (Bela-Pharm GmbH & Co. KG, Vechta, Germany). Intrastriatal stereotaxic transplantation was conducted by applying the microtransplantation technique (Nikkhah et al., 1994). Rats were classified in 2 groups: animals immunosuppressed with cyclosporine A, and control animals (sodium chloride). The animals received about 200,000 cells (a total of 4 μl suspension) into the right striatum by using a glass capillary with an outer diameter of 50–70 μm connected to a 5-μl Hamilton syringe. The coordinates with reference to the bregma were set as: $A = +1.3$, $L = +2.6$, and $V = +5.0$.

2.5. Tissue processing and analysis

Four and six weeks after transplantation respectively, the rats were injected with an overdose of pentobarbital (60 mg/kg) and transcardially perfused with 0.9% sodium chloride (100 ml), followed by 500 ml of 3.7% paraformaldehyde. Brains were immediately removed from the skull, postfixed for 3 h, and transferred into 0.1 M phosphate buffer containing 20% sucrose at 4 °C until sinking (1–3 days). The cryoprotected brains were frozen in isopentane (−50 °C) and stored at −80 °C until further processing. Brains were cut with a cryostat at 30 μm and sections were collected free floating in PBS. For diaminobenzidine (DAB) staining, the sections were pretreated with 3% H_2O_2, washed in PBS and blocked with PBS containing 5% normal goat serum and 0.3% Triton X-100 for 1 h at room temperature, and then incubated with mouse anti-Human Nuclei (HuNu, 1:400, Chemicon), mouse anti-Neuronal Nuclei (NeuN, 1:100, Chemicon) and rabbit anti-Glial Fibrillary Acidic Protein (GFAP, 1:500, DAKO) overnight at 4 °C. The following day, the sections were washed in PBS and incubated with biotinylated horse anti-mouse secondary antibody (1:200, Vector) or biotinylated goat anti-rabbit secondary antibody (1:500, Dianova) for 1 h at room temperature. After washing, the colour reaction for light microscopy was performed using first ABC solution (VectaStain Elite Kit, PK-6100, Vector) and then adding DAB Kit (Peroxidase Substrate Kit, SK-4100, Vector). The sections were then washed in PBS and mounted on superfrost slides. Mounted sections were dehydrated in graded alcohols, followed by incubation in xylene and finally embedded in Entellan.

For double immunofluorescence staining, blocking was performed with PBS containing 5% normal goat serum and 0.3% Triton X-100 (1 h). Sections were stained HuNu antibody (HuNu, 1:400, Chemicon) paired with anti-GFAP (1:500, DAKO). For antigen visualization goat polyclonal anti-mouse Cy3 (1:500, Dianova) and donkey anti-rabbit Cy2 (1:500, Dianova) were used. For