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Neuroprotective effects of human spinal cord-derived neural precursor cells after transplantation to the injured spinal cord

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ABSTRACT

To validate human neural precursor cells (NPCs) as potential donor cells for transplantation therapy after spinal cord injury (SCI), we investigated the effect of NPCs, transplanted as neurospheres, in two different rat SCI models.

Human spinal cord-derived NPCs (SC-NPCs) transplanted 9 days after spinal contusion injury enhanced hindlimb recovery, assessed by the BBB locomotor test. In spinal compression injuries, SC-NPCs transplanted immediately or after 1 week, but not 7 weeks after injury, significantly improved hindlimb recovery compared to controls. We could not detect signs of mechanical allodynia in transplanted rats.

Four months after transplantation, we found more human cells in the host spinal cord than were transplanted, irrespective of the time of transplantation. There was no focal tumor growth. In all groups the vast majority of NPCs differentiated into astrocytes.

Importantly, the number of surviving rat spinal cord neurons was highest in groups transplanted acutely and subacutely, which also showed the best hindlimb function. This suggests that transplanted SC-NPCs improve the functional outcome by a neuroprotective effect.

We conclude that SC-NPCs reliably enhance the functional outcome after SCI if transplanted acutely or subacutely, without causing allodynia. This therapeutic effect is mainly the consequence of a neuroprotective effect of the SC-NPCs.

Introduction

The last decade has seen an impressive number of articles on experimental cell therapies for spinal cord injury (SCI), involving embryonic stem cells, adult (Parr et al., 2008) and fetal (Ogawa et al., 2002) somatic neural stem cells, oligodendrocyte progenitor cells (Keirstead et al., 2005), umbilical cord blood cells (Cho et al., 2008), olfactory mucosa (Iwatsuki et al., 2008), different Schwann cell preparations (Paino et al., 1994) and olfactory ensheathing cells (Li et al., 1997). Several of these cell types are now used in on-going clinical trials, or have been approved for clinical trial by regulatory authorities. However, there are a depressing number of treatments that have shown effects in animal disease models but failed in clinical trials. We therefore set out to evaluate a promising human cell type in two very different animal models of SCI.

Neurospheres are free-floating heterogeneous cultures of immature neural precursor cells (NPCs) (Reynolds and Weiss, 1996), composed of neural stem cells, more committed progenitor cells, as well as occasional differentiated astrocytes and post-mitotic neurons (Piao et al., 2006). A number of studies have shown that there are differences between in vitro-expanded neural stem and progenitor cells due to their regional origins (Armando et al., 2007; Kelly et al., 2009; Onorati et al., 2011). Transplantation of NPCs, murine or human, has been shown to improve functional parameters in SCI animals (Iwanami et al., 2005; Ogawa et al., 2002; Watanabe et al., 2004). NPCs are therefore possible candidates for clinical application in SCI patients. It is however not clear if the region-dependent differences seen in neural stem and progenitor cells translate into different therapeutic effects in SCI.

Abbreviations: SCI, spinal cord injury; NPCs, neural precursor cells; SC-NPCs, spinal cord-derived NPCs; FBr-NPCs, forebrain-derived NPCs; GRPs, glial restricted progenitor cells; CNTF, ciliary neurotrophic factor; PFA, paraformaldehyde; PBS, phosphate-buffered saline; Hsp-27, heat shock protein 27; Hnp, human nuclear antigen; PCNA, proliferating cell nuclear antigen; GFAP, glial fibrillary acidic protein; MAP2, microtubule-associated protein 2; IR, immunoreactive.

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There are several possible mechanisms through which cell therapy may restore functions after SCI. Neuroprotection is one, the transplant acting by reducing endogenous toxic substances, inhibiting inflammatory reactions and/or releasing growth factors and other supportive substances (Madhavan et al., 2008; Yasuhara et al., 2006). Since the reversible degenerative processes after experimental SCI most likely proceed for a few weeks in rats, neuroprotection may be a mechanism through which acutely and subacutely transplanted cells act. In later stages, functional effects of transplanted cells may be due to the support of host axon re-growth through release of trophic factors, reduction of neurite growth-inhibitory substances, enhancement of re-myelination of axons, or by supplying the injured tissue with an extracellular matrix that is more permissive to regeneration. In rodent SCI models it is also possible that functional effects of a graft is due to modulation of the central pattern generators, without any improved supraspinal control of movements. In vitro studies have shown that NPCs have neuroprotective effects, which have also been suggested by in vivo studies (Mothe et al., 2013). Others have reported that these cells act by supporting regenerating host axons (Gamm et al., 2007; Pfeifer et al., 2004).

The timing of transplantation also has other implications. After measuring the levels of potentially cytotoxic cytokines, Nakamura and coworkers argued that the spinal cord is a hostile environment to transplanted cells the first week after injury (Nakamura et al., 2003). As a consequence, experimental transplantation studies are with few exceptions carried out 9 days after injury. However, there are to our knowledge no systematic comparisons between transplantations to the spinal cord at different time-points, a very important issue with regard to clinical applicability.

In the present study we have analyzed the functional effects of human spinal cord-derived NPCs (SC-NPCs) transplanted to SCI animals. To evaluate the potential of these NPCs as cell therapy for human SCI, we initially applied the NPC transplantation to dorsal contusion injuries with very rapid onset using the force-feedback IH impactor (Scheff et al., 2003). This was followed by a more extensive study using another type of SCI model, lateral compression injury, with a slow onset as a result of application of an aneurysm clip (von Euler et al., 1997), comparing NPC transplantation at different time points after injury. We found that human SC-NPCs improve functional outcome in both SCI models, but only after acute and subacute transplantations, and the functional improvement correlated with a neuroprotective effect of the transplanted NPCs, which reduced the loss of host spinal cord neurons.

Materials and methods

Animals

Adult immunodeficient athymic female rats (Hsd: RH-rnu/rnu, Harlan) were housed in an isolated environment, with autoclaved water and food pellets ad lib. The use of research animals was done in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Swedish Animal Protection Act, and the experimental procedures were approved by the Regional Ethics Committee on Animal Research, Stockholm, Sweden.

Human neurosphere culture

NPCs transplanted as neurospheres were derived from two types of donor tissue; SC-NPCs from the spinal cord, and FBr-NPCs from the subcortical forebrain, 5.5–9 weeks of gestation. The embryonic–fetal tissue was retrieved from elective routine abortions, with written consent from the pregnant women. All procedures for the use of human cells for experimental studies on SCI were approved by the Regional Ethical Committee, Stockholm. Tissue was dissected and cultured as earlier described by us (Piao et al., 2006). Briefly, dissection was made under sterile conditions in DMEM with F12 (DMEM/F12, 1:1, Life Technologies, Gaithersburg, MD, USA), and mechanical dissociation of the tissue was performed using a glass-Teflon homogenizer, using the entire spinal cord or sub-cortical forebrain of one fetus to establish NPC cultures. The cells were cultured as free-floating neurospheres in DMEM/F12 complemented with glucose (0.6%, Sigma, St. Louis, MO, USA), Hepes (5 mM, Life Technologies), heparin (2 μg/ml, Sigma), N_2 supplement (1%, Life Technologies), basic fibroblast growth factor (20 ng/ml, R&D Systems, Minneapolis, MN, USA), epidermal growth factor (20 ng/ml, R&D Systems) and ciliary neurotrophic factor (CNTF, 10 ng/ml, R&D Systems) at an initial density of 40,000–50,000 cells/cm^2 in 20 ml of medium. The NPCs were maintained at 37 °C in 5% CO_2, with addition of fresh medium twice a week. They were passaged every 7 to 10 days with enzymatic dissociation using TrypLE Express (Life Technologies) for 4–5 min at 37 °C followed by gentle mechanical dissociation.

Selection of neurospheres for transplantation

Using quantitative cell counts in immersion-fixed, sectioned medium-sized neurospheres, we established the equation $n = \exp(k \times s + m)$, $k = 0.009$ and $m = 6.26$ describing the relation between the diameter (s) and the number of cells in the neurosphere (n) for neurospheres with diameters between 100 and 500 μm.

Neurospheres at passage 3–8 were used for all experiments. In the neurosphere cultures, spheres with a diameter between 150 and 300 μm were identified, the number of cells per neurospheres calculated, and 10–12 neurospheres were chosen to give a total of 100,000 cells ± 10% to be transplanted to each rat. The neurospheres were kept in cell culture medium without growth factors pending transplantation.

Animal surgery

The weight of the rats was 170–200 g at the time of surgery. They were injected with Atropin (0.05 mg/kg i.p., NM Pharma AB) 30 min before surgery, and anesthetized using Hypnorm (fentanyl citrate, 0.22 mg/kg, and fluanisone, 6.8 mg/kg, Janssen Pharmaceutical) and Dormicum (midazolam, 3.4 mg/kg, Hoffman-La Roche). Body temperature was kept at 38 °C throughout surgery.

Lumbar spinal cords were surgically exposed by partial laminectomy of vertebra Th13, the dura was cut open, and a few drops of Xylocaine (lidocaine hydrochloride 20 mg/ml, AstraZeneca) were placed on the exposed spinal cord. Spinal cord contusion injury was achieved with an IH spinal cord impactor (Precision Systems and Instrumentation, LLC), the force set to 175 kdyn with no dwell time. For lateral compression injuries, a modification of the method of Rivlin and Tator (1978) was used. A bulldog clamp was applied to compress the spinal cord for 30 s at the lower half of the spinal cord segment Th13.

In acutely transplanted animals, NPCs were injected in the same session as the injury (see below). For the other animals, a layer of Lyoplant (B/Braun Aesculap AG) was placed on the spinal cord as dura substitution before the wound was sutured. The rats were subcutaneously injected twice with 3 ml Ringer/glucose (2.5%) before and after surgery. After surgery, the rats were given intramuscular injections of Temgesic (buprenorphine, 7 μg/kg, Reckitt & Colman) twice a day for four days to avoid allodynia, and Borgal (trimetoprim sulfa, 15 mg/kg s.c., Intervet International B.V.) to prevent from urinary infection. The urinary bladders were emptied manually twice daily until spontaneous evacuation was present.

Transplantation procedures

Transplantation to dorsal spinal cord contusion injuries was performed 9 days after lesion. Rats were randomized for transplantation, and either received 100,000 SC-NPCs or a 3 μl injection of growth factor-free cell culture medium as sham transplantation.

At the time of transplantation, animals were anesthetized, the wound re-opened and the spinal cord exposed. A glass capillary (0.3 mm end