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Research Report

Effects of human OEC-derived cell transplants in rodent spinal cord contusion injury

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ABSTRACT

Numerous reports indicate that rodent olfactory ensheathing cells (OECs) assist in spinal cord repair and clinical trials have been undertaken using autologous transplantation of human olfactory ensheathing cells (hOECs) as a treatment for spinal cord injury. However, there are few studies investigating the efficacy of hOECs in animal models of spinal cord injury. In this study hOECs were derived from biopsies of human olfactory mucosa, purifed by culture in a serum-free medium containing neurotrophin-3, genetically labelled with EGFP, and stored frozen. These hOEC-derived cells were thawed and transplanted into the spinal cord injury site 7 days after a moderate contusion injury of the spinal cord at thoracic level T10 in the athymic rat. Six weeks later the animals receiving the hOEC-derived transplants had greater functional improvement in their hindlimbs than controls, assessed using open field (BBB scale) and horizontal rung walking tests. Histological analysis demonstrated beneficial effects of hOEC-derived cell transplantation: reductions in the volume of the lesion and the cavities within the lesion. The transplanted cells were located at the periphery of the lesion where they integrated with GFAP-positive astrocytes resulting in a significant reduction of GFAP staining intensity adjacent to the lesion. Although their mechanism of action is unclear we conclude that hOEC-derived cell transplants improved functional recovery after transplantation into the contused spinal cord, probably by modulating inflammatory responses and reducing secondary damage to the cord.

1. Introduction

Traumatic spinal cord injury is a complex, progressive event leading to long-term motor and sensory deficits, and dysfunction of the autonomic nervous system. In the spinal cord there is cell death, severing of axons, demyelination, inflammation and the formation of cystic cavities (Sekhon and Fehlings, 2001). There is limited capacity of the human spinal cord to repair itself, so there is a need for therapeutic strategies to protect and repair the injured spinal cord. Olfactory ensheathing cells (OECs) are a unique type of glial cell that share some properties with astrocytes and Schwann cells but have

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Abbreviations: OEC, olfactory ensheathing cell; GFAP, glial fibrillary acidic protein; BBB, Basso Beattie Bresnahan locomotor rating scale; pFB-hrGFP, green fluorescent protein

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intrinsic properties that make them ideal candidates for cellular grafts into injured spinal tissue (Chuah and Au, 1994; Fairless and Barnett, 2005; Farbman and Squinto, 1985; Ramon-Cueto and Valverde, 1995).

Many studies have demonstrated that OECs are therapeutic when transplanted into the injured spinal cord in rodent models including restoration of motor function (Keyvan-Fouladi et al., 2003; Kubasak et al., 2008; Li et al., 2003; Li et al., 1997; Lopez-Vales et al., 2007), improvement of respiratory function (Li et al., 2003) and restoration of bladder function (Pascual et al., 2002). Transplanted OECs induced axon regeneration (Keyvan-Fouladi et al., 2003; Li et al., 1997, 1998, 2007; Lopez-Vales et al., 2007; Plant et al., 2003; Ramon-Cueto et al., 1998), remyelination of axons (Barnett et al., 2000; Imaizumi et al., 1998; Kato et al., 2000), reduced lesion size, cystic cavitation and astrocytic gliosis (Lopez-Vales et al., 2006; Ramer et al., 2004; Takami et al., 2002; Verdu et al., 2001) and ameliorated electrical conduction through the injury site (Imaizumi et al., 1998, 2000; Lopez-Vales et al., 2006, 2007; Toft et al., 2007).

Most previous studies have investigated the therapeutic properties of rat OECs although human OECs remyelinated spinal cord axons after demyelinating lesions (Barnett et al., 2000; Kato et al., 2000) and primate OECs induced locomotor recovery after transection injury in the rat (Guest et al., 2008). There are no previous studies of human OEC transplantation into the contused rat spinal cord, a model of injury that mimics aspects of many common human injuries. Most previous studies, including the human and primate reports, obtained OECs from the olfactory bulbs within the cranial cavity. This is not a clinically favoured site for sourcing autologous OECs because of the morbidity associated with surgical access and destruction of the olfactory bulbs to obtain the cells. OECs can be obtained from the olfactory mucosa in the nose (Bianco et al., 2004) and OECs from this source were therapeutic when transplanted into the transected spinal cord in rat (Lu et al., 2001, 2002). hOECs derived from the olfactory mucosa were similar to rat nasal OECs in proliferation, survival and migration after transplantation into the normal spinal cord of athymic rats (Deng et al., 2006). For autologous transplants, OECs are relatively easy to obtain from the human nose by biopsy of the olfactory mucosa, without affecting olfactory function (Feron et al., 1998). A Phase I clinical trial demonstrated that transplantation of autologous OECs was safe in human paraplegia (Feron et al., 2005; Mackay-Sim et al., 2008).

The aim of this study was to establish whether OECs obtained from the human mucosa and genetically labelled with green fluorescent protein are able to produce similar behavioural outcomes to that reported for rat OECs. While the remyelination potential of human bulbar OECs has been documented, this is the first time locomotor improvements following human mucosal OEC transplants have been investigated. We used the traumatic contusion model (MASCIS impactor) which results in a complex central lesion with damage to spinal cord tissue and blood vessels, and ongoing secondary injury and cyst formation (Basso et al., 1996) similar to human spinal injury (Sekhon and Fehlings, 2001). The use of athymic rats (Rolstad, 2001) limits xenograft rejection of the hOECs, and delaying transplantation until a week after injury reduces exposure of the grafted cells to the acute inflammatory phase.

2. Results

2.1. Sample

Twenty animals (9 receiving hOECs and 11 controls) were used in the final analysis. Three animals were excluded from the study because the compression rate during spinal cord contusion and/or the initial BBB score was more than two standard deviations from the group mean and one animal was excluded due to post-operative complications. The mean compression rate for the hOEC transplanted group was 0.36±0.02 m/s compared to 0.38±0.04 m/s for the control group. There was no difference between the groups ($t=0.658$, df 18, $p=0.52$). The lesion locations were comparable for the transplanted and non-transplanted group lesions. The positioning of the impactor head between the T9 and T11 vertebrate prevents much variation in lesion location between animals. There was a small decrease in body weight (<10%) in the first week after the surgery that then increased during the 6-week post-contusion period to above baseline levels. All rats were spontaneously voiding urine by 2 weeks post-contusion surgery. The cell transplantation procedure 1 week after spinal cord contusion did not cause any noticeable adverse effects. Despite obvious locomotor deficits, all animals were alert, healthy and mobile after surgery.

2.2. Phenotyping of cells

Prior to transduction with pFB-hrGFP, cells were identified immunocytochemically with antibodies against glial fibrillary acidic protein (GFAP; Dako, Denmark), S100 (Dako, Denmark), $p75^{NTR}$ (Neubody, AUS) and R-phycoerythrin-conjugated anti-HNK1 (Sigma). The percentages of S100-positive and GFAP positive cells were >95%, nearly all cells were also $p75^{NTR}$ immunoreactive and there was no staining of NHK 1 antibody (Feron et al., 2005) indicating the culture was highly enriched for OEC. However immunophenotyping of these cells just prior to transplantation showed no expression of any of these markers. For reasons addressed in Discussion, we consider the most likely explanation for this loss of expression is due to plasticity of the OECs which is well known to occur in vitro (Barnett et al., 2000; Vincent et al., 2003, 2005; Moreno-Flores et al., 2003), rather than replacement of all the OECs by another immunonegative cell type. We therefore have used the term hOECs to describe these transplants, but acknowledge their identity is unconfirmed.

2.3. Behavioural assessments

2.3.1. Open field assessment

During the 6 weeks following hOEC transplantation or medium injection there was an increase in BBB scores for both groups (Fig. 1A) with a significantly greater improvement in the hOEC transplant group, (two way ANOVA repeated measures, F (1,18)=9.6, $p=0.006$). Bonferroni post hoc tests show significant increases in the OEC group by 4 weeks post