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Olfactory ensheathing cells but not fibroblasts reduce the duration of autonomic dysreflexia in spinal cord injured rats

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ABSTRACT

Autonomic dysreflexia is a common complication after high level spinal cord injury and can be life-threatening. We have previously shown that the acute transplantation of olfactory ensheathing cells into the lesion site of rats transected at the fourth thoracic spinal cord level reduced autonomic dysreflexia up to 8 weeks after spinal cord injury. This beneficial effect was correlated with changes in the morphology of sympathetic preganglionic neurons despite the olfactory cells surviving no longer than 3 weeks. Thus the transitory presence of olfactory ensheathing cells at the injury site initiated long-term functional as well as morphological changes in the sympathetic preganglionic neurons. The primary aim of the present study was to evaluate whether olfactory ensheathing cells survive after transplantation within the parenchyma close to sympathetic preganglionic neurons and whether, in this position, they still reduce the duration of autonomic dysreflexia and modulate sympathetic preganglionic neuron morphology. The second aim was to quantify the density of synapses on the somata of sympathetic preganglionic neurons with the hypothesis that the reduction of autonomic dysreflexia requires synaptic changes. As a third aim, we evaluated the cell type-specificity of olfactory ensheathing cells by comparing their effects with a control group transplanted with fibroblasts. Animals transplanted with OECs had a faster recovery from hypertension induced by colorectal distension at 6 and 7 weeks but not at 8 weeks after T4 spinal cord transection. Olfactory ensheathing cells survived for at least 8 weeks and were observed adjacent to sympathetic preganglionic neurons whose overall number of primary dendrites was reduced and the synaptic density on the somata increased, both caudal to the lesion site. Our results showed a long term cell type-specific effects of olfactory ensheathing cells on sympathetic preganglionic neurons morphology and on the synaptic density on their somata, and a transient cell type-specific reduction of autonomic dysreflexia.

1. Introduction

Cardiovascular disorders after spinal cord injury represent a leading cause of morbidity and mortality in spinal cord injured individuals (Garshick et al., 2005; Myers et al., 2007; Soden et al., 2000). Of particular clinical relevance is autonomic dysreflexia which is characterized by a sudden and massive generalized discharge of sympathetic preganglionic neurons below the injury site leading to extremely high arterial blood pressure. Untreated episodes of autonomic dysreflexia may have serious consequences including epileptic seizures, retinal detachment, stroke and even death, reviewed in (Wan et al., 2014).

Olfactory ensheathing cells (OECs) represent one of the most promising candidates for cell-mediated repair following spinal cord injury. Despite variability in replication, numerous studies have shown that OECs can promote a wide range of beneficial effects including neural repair, tissue sparing, neuroprotection, decreasing cavity formation, modification of the glial scar, angiogenesis and remyelination of demyelinated axons (Franssen et al., 2007; Mackay-Sim et al., 2011; Raisman et al., 2012; Raisman et al., 2007; Richter et al., 2008; Roet et al., 2014; Yang et al., 2015). OEC transplantation was also beneficial in a rat model of autonomic dysreflexia resulting from spinal cord transection at the fourth thoracic spinal cord level (T4) (Kalincik et al., 2010a). Acute transplantation of OECs at the lesion site reduced the duration of autonomic dysreflexia and changed the morphology of spinal cord sympathetic preganglionic neurons that play a crucial role in central cardiovascular control (Cabot, 1996; Calaresu et al., 1988; Chalmers et al., 1991; Reis et al., 1988). These sympathetic

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preganglionic neurons are particularly important for autonomic dysreflexia in the T4-transected rat model (Leman et al., 2002). After T4 spinal cord transection there was a sustained increase in soma size of sympathetic preganglionic neurons with a temporary increase in the number and lengths of their primary dendrites (Kalincik et al., 2010b). OEC transplantation into the lesion site normalized the changes in soma size of the sympathetic preganglionic neurons below the lesion, while increasing the lengths of dendrites in the sympathetic preganglionic neurons above the lesion (Kalincik et al., 2010a; Kalincik et al., 2010b). These increases in soma sizes persisted for 9 weeks after OEC transplantation but the increases in dendrite number and length were only temporary (Kalincik et al., 2010b). For these experiments the OECs were transplanted into the lesion site immediately after the spinal cord transection. The OECs survived no >3 weeks indicating that the transitory presence of OECs at the injury site initiated long-term functional as well as morphological changes. This outcome may be further improved if the transplanted OECs survived for longer, for example by transplanting them into the spinal cord adjacent to the lesion site and not into its epicenter (Pearse et al., 2007). The first aim of the present study was to evaluate whether OECs survive after transplantation within the parenchyma close to sympathetic preganglionic neurons and whether, in this position, they still reduce the duration of autonomic dysreflexia and modulate sympathetic preganglionic neuron morphology.

The severity of attacks of autonomic dysreflexia after T4/5 spinal cord transection is believed to involve a reorganization of synaptic circuitry controlling the activity of sympathetic preganglionic neurons (Llewellyn-Smith, 2009; Maiorov et al., 1997a; Maiorov et al., 1997b; Marsh et al., 2004). Therefore the reduction in severity of autonomic dysreflexia by OEC transplantation may involve a reorganization in synaptic inputs as well as changes in the morphology of sympathetic preganglionic neurons. The second aim of the present study was to quantify the density of synapses on the somata of sympathetic preganglionic neurons after OEC transplantation with the hypothesis that the reduction of autonomic dysreflexia requires synaptic changes as well as the morphologic changes as previously observed (Kalincik et al., 2010a; Kalincik et al., 2010b).

Previous studies have demonstrated the cell type-specificity effects of olfactory ensheathing cells by comparing them with a fibroblasts transplanted group as a control (Khankan et al., 2016; Toft et al., 2013). As a third aim, we sought to confirm that the changes in autonomic dysreflexia, sympathetic preganglionic neurons morphology and the density of synapses on the somata of sympathetic preganglionic neurons were specific to olfactory ensheathing cells and not induced by fibroblasts (control group) as a generalized response to cell transplantation.

2. Materials and methods

2.1. Animals

Experiments were performed on 24 adult male Australian inbred Wistar rats (Biological Resource Centre, Sydney, New South Wales) (350–400 g). They were housed in individual plastic home boxes (65 × 40 × 22 cm) with ad libitum food and water and maintained on a 12/12 h light/dark cycle. All experimental procedures were approved by the Animal Care and Ethics Committees of the University of New South Wales and Griffith University in accordance with guidelines of the National Health and Medical Research Council of Australia.

2.2. Biopsy and culture of olfactory ensheathing cells and fibroblasts

Six adult male albino Australian inbred Wistar rats (Biological Resource Centre, Sydney, New South Wales) (350–400 g) were used for harvesting and culture of OECs and fibroblasts. These rats were deeply anaesthetized with pentobarbitone (0.5 ml intraperitoneally; Lethabarb, Virbac Pty. Ltd., NSW, Australia) and killed by decapitation. OECs were obtained from the olfactory mucosa of rats in the same manner as previously described (Bianco et al., 2004). Isolation and purification of the OECS were performed in the same manner as previously described (Kalincik et al., 2010a). Primary cultures of fibroblasts from the same strain of rat were generated from skin biopsies according to published protocol (Matigian et al., 2008). Only OECs were modified to express humanised *Renilla* green fluorescent protein as described previously (Gorrie et al., 2010). OECs and fibroblasts were cryopreserved from tissue culture flasks as described previously (Gorrie et al., 2010).

2.3. Spinal cord transection and cell transplantation

Eighteen rats were anaesthetised with a mixture of ketamine hydrochloride (100 mg kg^{-1} intraperitoneally; Ketamine, Parnell Laboratories Pty. Ltd., NSW, Australia) and xylazine (7 mg kg^{-1}, intraperitoneally; Ilium Xylazil-20, Troy Laboratories Pty. Ltd., NSW, Australia). Laminectomy of the T3 vertebra was performed to expose the T4 spinal segment like described previously (Kalincik et al., 2010a). After immobilization of the spinal process rostral to the laminectomy site, GFP-OECs (n = 9 animals) were stereotaxically injected using a sterile 10 μl Hamilton syringe (Hamilton, Reno, NV) with a silicon-coated pulled glass tip (with inner diameter of 80–100 μm). Rats (n = 9) received four injections of GFP-OECs (0.5 μl of 100,000 cells/μl, in culture medium DMEM/F12), two injections 2 mm cranial and two 2 mm caudal to the future lesion site, each 0.5 mm lateral to the midline at 0.8 mm deep. For each injection, the needle was left in situ for 1–2 min to prevent reflux. Control animals (n = 9) received fibroblasts at similar concentrations. The spinal cord was then completely transected with microscissors between the two pairs of injection sites. Complete transection was confirmed with a scalpel blade and observation under the dissecting microscope. Following transection, a gap of approximately 1–2 mm was present between the rostral and caudal ends of the cord and a piece of absorbable gelatin sponge (SPONGOSTAN®, Johnson and Johnson medical limited, Sipton, U.K.) was inserted to fill the gap and reduce bleeding. Another piece of absorbable gelatin sponge was also placed on the dorsal surface of the cord and the muscle tissue and skin were sutured in layers.

Immediately after surgery, the rats received the muscarinic cholinoceptor antagonist atropine methyl nitrate (3 mg kg^{-1} subcutaneously; Sigma, St. Louis, MO, USA). An antibiotic (Cephalothin, 30 mg kg^{-1} subcutaneously; VIC, Australia) was also administered twice daily until there was no sign of urinary infection (noted by cloudiness of expressed urine) for three consecutive days. Animals also received an analgesic (Carprofen, 50 mg kg^{-1} subcutaneously; Australia Pty. Ltd., Australia) daily for 2–3 days. Lactated Hartmann's solution (5–10 ml subcutaneously; Baxter Healthcare Pty. Ltd. Sydney, Australia) was administered 2–3 times daily until the rats were able to drink normally. Manual emptying of the urinary bladder was carried out 3 times a day until the rats developed a micturition reflex (~2 weeks). The room temperature was maintained at 27–28 °C for the first 1–2 weeks and rat core temperature was measured regularly until normal temperature was maintained. The room temperature was maintained at 26 °C until the end of the experiment.

2.4. Implantation of radio-telemetric probes

Four weeks after spinal cord transection, the rats were chronically implanted with radio-telemetric probes (PA-C40, Data Sciences International St. Paul, MN, USA.) for the measurement of the mean arterial pressure and heart rate as described previously (Cloutier et al., 2010).