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Research report

Olfactory ensheathing glia cell therapy and tubular conduit enhance nerve regeneration after mouse sciatic nerve transection

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ABSTRACT

The regenerative potential of the peripheral nervous system (PNS) is widely known, but functional recovery, particularly in humans, is seldom complete. Therefore, it is necessary to resort to strategies that induce or potentiate the PNS regeneration. Our main objective was to test the effectiveness of Olfactory Ensheathing Cells (OEC) transplantation into a biodegradable conduit as a therapeutic strategy to improve the repair outcome after nerve injury. Sciatic nerve transection was performed in C57BL/6 mice; proximal and distal stumps of the nerve were sutured into the collagen conduit. Two groups were analyzed: DMEM (acellular grafts) and OEC ($1 \times 105/2$ μL). Locomotor function was assessed weekly by Sciatic Function Index (SFI) and Global Mobility Test (GMT). After eight weeks the sciatic nerve was dissected for morphological analysis. Our results showed that the OEC group exhibited many clusters of regenerated nerve fibers, a higher number of myelinated fibers and myelin area compared to DMEM group. The G-ratio analysis of the OEC group showed significantly more fibers on the most suitable sciatic nerve G-ratio index. Motor recovery was accelerated in the OEC group. These data provide evidence that the OEC therapy can improve sciatic nerve functional and morphological recovery and can be potentially translated to the clinical setting.

1. Introduction

Peripheral nerve injuries occur in approximately 2.8% of trauma patients (Noble et al., 1998). The regenerative potential of the peripheral nervous system (PNS) is widely known, but functional recovery, particularly in humans, is seldom complete. Furthermore, when there is extensive tissue loss after injury or when the distance between the lesion and the target tissue is too large, the regenerative process is restricted or even blocked (Boyd and Gordon, 2003). In these cases, it is necessary to resort to strategies that induce or potentiate the regeneration of nerve fibers. Various strategies have been used, with focus on: 1) Autologous grafts of cutaneous nerves; 2) Use of a tubular conduits (Langone et al., 1995); 3) Gene therapy associated with conduits (Pereira Lopes et al., 2011); 4) Cell therapy associated with conduits (Lopes et al., 2006; Oliveira et al., 2010); 5) Physical therapy and exercise (Deumens et al., 2010); 6) Cell therapy associated with the conduits and exercise (Goulart et al., 2014). All these strategies have limitations and when used isolated they do not promote full functional recovery. Therefore, there is a continuing search for new techniques that can optimize the regeneration and functional recovery, after peripheral nerve injury.

The use of tubular conduit is capable of enhance the regeneration process, guiding axonal growth, isolating, protecting and maintaining trophic factors in the injury site (Chamberlain et al., 1998; Jang et al., 2016; Safa and Buncke, 2016). Among the several types, biodegradable tubes have shown major application in basic and clinical research, because its nature provides biocompatibility, and decrease the probability of an immune response (Muheremu and Ao, 2015). Additionally, the tubulization technique leads to promising results when associated with cell therapy (Liao et al., 2016; Midha et al., 2003; Pereira Lopes et al., 2011).

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New prospects for promoting nerve regeneration after injury emerge from the use of cell therapies. The olfactory ensheathing cells (OEC) are distributed to the nasal mucosa, nerve and olfactory bulb, and are especially interesting because they are associated with the axons of olfactory neurons from the nasal mucosa in the PNS, to the olfactory bulb in the CNS (Ekberg and St John, 2015; Su et al., 2013). Furthermore, the olfactory system is one of the places of highest degree of plasticity in the CNS, where there is a continuous neurogenesis process of the olfactory epithelium (Gorrie et al., 2010; Mariano et al., 2015). This neurogenesis is a process highly regulated and assisted by OEC (Ingram et al., 2016). Therefore, olfactory ensheathing cells are called "Schwann cell-like" due to its ability to promote axonal growth and "Astrocyte-like" because they reside in the CNS (Khankan et al., 2015; Vadivelu et al., 2015). Although the OEC are great candidates to promote repair after a nerve injury, the mechanisms involved in this process remain largely unidentified (Rela et al., 2015; Zhao et al., 2015).

In vitro and in vivo studies have shown that OEC can enhance neurite elongation in a scar-like culture model and after spinal cord injury, respectively (Khankan et al., 2015; Kubasak et al., 2008). Experimental in vivo studies inducing neurological damage by chemical and / or mechanical methods suggested that the OEC isolated from mice and transplanted into regions close to the injury can improve axonal growth and remyelination of axons newly lengthened (Dombrowski et al., 2006; Li et al., 2003; Ramón-Cueto and Nieto-Sampedro, 1994). Additionally, OEC transplantation has been described as being responsible for increasing the regeneration of peripheral nerve and improving functional outcome after nerve microsurgical repair in rats (Kabiri et al., 2015; Penna et al., 2012; Radtke et al., 2009).

The present study tested the effectiveness of OEC transplantation into a biodegradable conduit as a therapeutic strategy to improve the outcome of repair after mouse nerve injury. Our findings suggest that this therapeutic strategy can significantly improve functional and morphological recovery.

2. Results

2.1. Cultured OEC express CNPase and SMActin

At visual inspection, mouse OEC cultures washed with medium, fixed with paraformaldehyde and reacted with anti-CNPase and anti-SM α-actin seemed to contain no unlabeled cells or cells labeled with one marker only. Our estimate of the purity of cells was based on a previous study of one of us (unpublished data). In that study, cultures from the olfactory fiber layer were stained with anti-CNPase and anti-GFAP. Mosaics containing 100 pictures taken with a microscope (AxioImager M2/ Carl Zeiss), with 20X/0.8 objective were shown to 5 naïve observers and these were asked to look at the pictures and indicate how many cells were (a) unlabeled or (b) labeled with only one of the markers or (c) apparently labeled with both markers but presenting a noticeable difference in the intensity of labeling of the two markers. Cells characterized as (a)+(b)+(c) were judged to be in the range of 1–5%. Thus, it seems that our method is highly effective for OEC enrichment (Fig. 1A-D).

2.2. Combined cell therapy improves nerve regeneration morphologically

The therapy with Olfactory Ensheathing Cells improved nerve

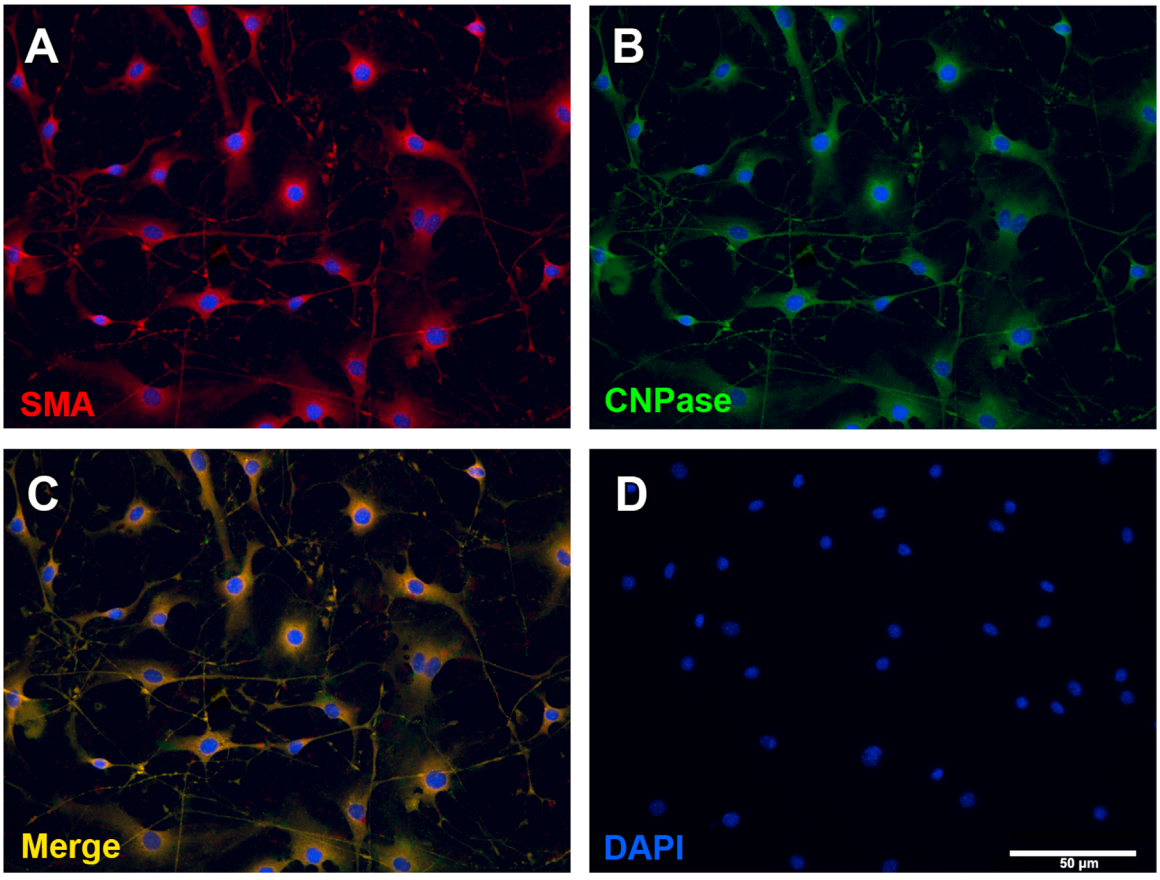

Fig. 1. Immunohistochemistry of olfactory ensheathing cells in culture. (A) Cells expressing SMA, a protein present in the OEC cells and absent in the Schwann cells. (B) Olfactory ensheathing cells expressing CNPase. (C) Image showing the co-localization of SMA, CNPase and DAPI. (D) Cell nuclei are highlighted by DAPI nuclear marker.