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Functional and regenerative effects of local administration of autologous mononuclear bone marrow cells combined with silicone conduit on transected femoral nerve of rabbits

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ABSTRACT

The inoculation of cells into injury sites can accelerate and improve the quality of nerve regeneration. This study aimed to evaluate the functional and regenerative effects of mononuclear autologous bone marrow cells (MABMC) combined with silicon conduit grafting in rabbit femoral nerves. Twenty-eight animals were allocated to one of two groups: treatment group (TG) or control group (CG), divided according to the time of evaluation, at either 50 or 75 days. After neurotmesis of the femoral nerve, surgical repair was performed with nerve autografts in silicon conduits, leaving a 5 mm gap in both groups. The TG received MABMC in silicon conduits, and CG received a sham saline inoculum. Histological, clinical and electrophysiological analyses detected no differences between groups, but analysis of leg diameter showed that TG diameters were larger. This cell therapy did not improve regeneration of the femoral nerve, but there was a tendency for better functional recovery.

1. Introduction

The peripheral nerves are susceptible to all of the same types of traumas that other tissues can suffer, but when the continuity of a nerve structure is interrupted, transmission of nervous impulses is stopped and functional activities are disrupted (Mattar and Azze, 2008) causing considerable disability and/or permanent physical incapacity (Ignatiadis et al., 2007). This is why there are scientists all over the world conducting research designed to increase the understanding of the nerve regeneration process and employing a variety of treatment approaches in attempts to find the ideal method with which injured nerves can be restored to full functionality (Santos and André, 2007).

There are reports of successful surgical repair of peripheral nerves in literature going back to the nineteenth century (Ignatiadis et al., 2007), when suggested that direct repair of nerves could be achieved by drawing together the epineurium. However, not all nerve injuries provide the conditions for direct anastomosis and significant nerve tissue losses demand grafts or conduits allowing communication between the two extremities (Mimura et al., 2004; Ignatiadis et al., 2007). These conduits or regeneration guides are made from biological or synthetic materials and are considered viable alternatives, for grafting techniques, performing the function of helping to guide axonal growth from a sectioned nerve, containing the diffusion of neurotrophic and neuroprotective factors with regenerative functions produced by the nerve stumps (Murakami et al., 2003; Yin et al., 2007; Zhang et al., 2008; Salomone et al., 2013). Even using these techniques, regeneration is still a slow process and one that is not always completed, since the silicone remains within the body and can cause foreign body reactions and may compress the nerve, leading to loss of function and formation of a neuroma (Yin et al., 2007). In recent attempts to achieve recuperation of both nerve continuity and function, a treatment combining cell therapy and trophic factors with the well-established technique of nerve repair using tubes has been tested (Chen et al., 2007; Colomé et al., 2008; Wang et al., 2011; Costa et al., 2013; Mohammadi et al., 2013) with encouraging results in terms of nerve regeneration.

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The objective of this study was to evaluate the functional and histological effects of a combination of silicone tube conduit and inoculation of autologous mononuclear bone marrow cells on rabbit femoral nerves after neurotmesis.

2. Materials and methods

2.1. Animals

A total of 28 New Zealand rabbits (*Oryctolagus cuniculus*) of both sexes (17 females and 11 males), aged 4 ± 1 months and with body mass of 3.52 kg ± 0.64 were obtained from the Universidade Federal de Santa Maria (UFSM) central animal house.

The animals were given a period of at least 5 days to acclimatize at the Hospital de Clínicas de Porto Alegre (HCPA) animal experimentation unit where they were fed on a commercial rabbit food in pellets and water ad libitum and housed in individual cages at a controlled temperature of 18.9 °C with mean air humidity of 68.2% and a 12 + 12 light–dark cycle.

The animals were allocated to one of two equal sized groups at random: Control (CG, $n = 14$) or Treatment (TG, $n = 14$), which were then further subdivided into two equal subsets for evaluation at either 50 or 75 days (CG50, $n = 7$; TG50, $n = 7$; CG75, $n = 7$; TG75, $n = 7$).

This study was reviewed by the HCPA Animal Research Ethics Committee for compliance with the principles and standards regulating the use of experimental animals and was approved under protocol number 07672.

2.2. Harvesting and processing autologous mononuclear bone marrow cells

Bone marrow was harvested in advance of the surgical procedure, under sterile conditions and all samples were handled separately.

Autologous mononuclear bone marrow cells were extracted from the greater tubercle of the humerus of both limbs or until a minimum bone marrow aspirate volume of 5 mL had been collected. Animals were anesthetized with ketamine hydrochloride (20 $mg \cdot kg^{-1}$), midazolam (0.5 $mg \cdot kg^{-1}$) and pethidine hydrochloride (5 $mg \cdot kg^{-1}$) via intramuscular (i.m) injection and then isoflurane vaporization in 100% oxygen was initiated. All of the animals were given intravenous (i.v) enrofloxacin (5 $mg \cdot kg^{-1}$) at the point of anesthetic induction.

Bone marrow aspirate was homogenized and washed twice with D-MEM culture medium containing 10% fetal bovine serum and 1% penicillin and then added to conical tubes containing Ficoll-hypaque. The resulting cell suspension was centrifuged for 5 min at 160 ×*g* (Eppendorf®, USA).

The cell pellet was resuspended in 3 mL of complete D-MEM medium. Another conical tube was prepared by adding 3 mL of Ficoll-hypaque (proportion 1:1) and then the cell suspension was pipetted onto the gradient, down the side of the tube, before the tube was centrifuged for 20 min at 110 ×*g* and 18 °C. After centrifugation, the mononuclear cells at the interface were removed once more, placed in another conical tube and centrifuged for a further 5 min at 200 ×*g*. The cell pellet was then resuspended in 1 mL of PBS. Cells were quantified and tested for viability using trypan blue vital stain.[1]

2.3. Cell transplantation

A total of 1×10^6 viable cells in a volume of 0.2 mL were transplanted the same day, into the silicone prosthesis, which was itself attached to the right femoral nerve that had been transected. For cell transplantation, it was used a needle 12.7 mm × 33 mm (29 G) attached in a syringe and administered through the distal end of the silicone tube.

[1] Trypan blue, Acros Organic, Geel, Belgium.

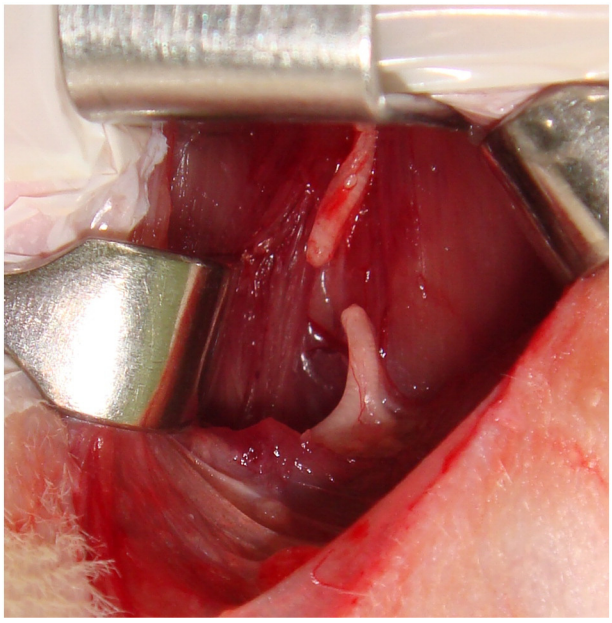

Fig. 1. Nerve femoral of rabbit. Location of the femoral nerve and nerve section.

2.4. Surgical procedure and transplantation of autologous mononuclear bone marrow cells

With the animal positioned in decubitus dorsal and under general anesthetic, a transverse incision was made at the right groin. The femoral nerve was located and completely sectioned, without removing any portion of the nerve (Fig. 1). A 7.5 mm long section of hollow cylindrical silicone tubing,[2] with internal diameter of 1.5 mm and external diameter of 2.42 mm was fitted over the extremities of the sectioned nerve. This was achieved by placing the tube between the nerve stumps and then sliding the proximal stump into the tube and suturing it to the epineural tissues with a single simple stitch using 6–0 nylon monofilament thread. The procedure was repeated for the distal stump, leaving a gap of approximately 5 mm between the nerve extremities. A surgical microscope[4] was used to aid suturing with 40× image magnification.

In the TG, a volume of 0.2 mL containing 1×10^6 mononuclear autologous cells, previously harvested from bone marrow aspirate was inoculated into the space within the silicone tube. In the CG, 0.2 mL of 0.9% NaCl solution was used instead (Fig. 2).

At the end of surgery and for the 2 following days, all rabbits were given ketoprofen[3] (1.0 $mg \cdot kg^{-1}$ IM, SID). They were also given tramadol hydrochloride[4] (2.5 $mg \cdot kg^{-1}$ IM, SID) for 5 days and enrofloxacin[5] was used as systemic antibiotic therapy at a dosage of 5 $mg \cdot kg^{-1}$ IM, SID, for the first 5 postoperative days. The surgical wound was cleaned using 0.9% NaCl solution every 24 h until healed.

2.5. Clinical assessment

After the date of the surgical procedure (day zero) animals were assessed clinically every 10 days. The area innervated by the femoral nerve was tested for sensitivity using a needle and animals were also tested for conscious proprioception. The thickness of both pelvic limbs was measured using tape measure. Finally, the animals' ambulatory ability was assessed before and after the nerve section procedure,

[2] Medicone, Cachoeirinha, RS, Brazil.
[3] Ketofen, Rhodia, Mérieux, Paulínia, SP, Brazil.
[4] Tramadol, Cristália Produtos Químicos Farmacêuticos LTDA, Itapira, SP, Brazil.
[5] Flotril, Indústria Química e Farmacêutica Schering-Plough S/A, Rio de Janeiro, RJ, Brazil.