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Research Paper
Nerve Regeneration

Uncultured undifferentiated adipose-derived nucleated cell fractions combined with inside-out artery graft accelerate sciatic nerve regeneration and functional recovery

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Abstract. Effects of transplantation of adipose-derived nucleated cell fractions (ADNCs) on sciatic nerve regeneration were studied. A 10-mm sciatic nerve defect was bridged using artery graft filled with ADNCs. In control group, artery graft was filled with saline alone. Regenerated nerve fibres were studied for 12 weeks. In sham-operated group, sciatic nerve was only exposed and manipulated. Behavioural and functional studies confirmed faster recovery of regenerated axons in ADNCs transplanted animals than in control group ($P < 0.05$). At the end of study period, animals in ADNCs transplanted group achieved a sciatic functional index (SFI) value of -31.6 ± -3.14, whereas in control group a value of -42.5 ± -3.7 was found. Gastrocnemius muscle mass in ADNCs transplanted animals was found to be significantly higher than that in control group ($P = 0.001$). Morphometric indices of regenerated fibres showed the number and diameter of myelinated fibres to be significantly higher in ADNCs transplanted animals than in control group ($P = 0.001$). On immunohistochemistry, there was more positive staining of S100 in the ADNCs transplanted animals than in control group. ADNCs transplantation into an artery graft could be considered a readily accessible technique that improves functional recovery of sciatic nerve.

Key words: adipose-derived nucleated cells; sciatic nerve repair; artery graft.

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Various techniques are used to achieve maximum functional recovery. In recent decades, the regenerative properties of stem cells have been investigated for use in nerve repair.[1] A method widely accepted by most surgeons is the bridging of the defect with an autologous donor nerve. Different graft equivalents have also been applied to bridge the nerve stump, regulated through the interaction of a variety of protein and cell signals.[2]

The conduits provide a guidance path for axons sprouting from the regenerating nerve end and provide a microenvironment for diffusion of neurotrophic and neurotropic factors secreted by the injured nerve stump, and hence help protect against infiltration of fibrous tissue.[3] An artery graft presents large quantities of laminin and some collagen. These substances are also found in Schwann cell basal membranes and could act as axonal outgrowth factors.[4–7] Laminin, one of the main basal membrane components, encourages neurite outgrowth, induces Schwann cell mitosis, and plays a crucial role in peripheral nerve repair.[8] As well as requiring nerve fibre contact, normal Schwann cell differentiation requires contact with a connective tissue matrix or some associated material such as collagen.[4] The standard artery graft basal membrane tube diameter is large, and the contact surface for migrating Schwann cells or axonal outgrowth cone adhesions becomes very small. Nevertheless, in inside-out artery grafts, this negative effect might be diminished once the adventitia provides a permissive matrix, which increases the contact surface for axons.[9]

The original and classical view of adipose tissue as a rather specialized passive storage organ has changed dramatically.[10] The adipose tissue has several properties that are advantageous for neuronal sprouting and direction, and has been used in different areas of surgery in recent decades.[11,12] Apart from adipocytes and pre-adipocytes, adipose tissue contains microvascular endothelial cells, smooth muscle cells, resident monocytes, lymphocytes, and stem cells.[13] In the last few years, it has been revealed that adipose tissue possesses a population of multi-potent stem cells that can differentiate into a Schwann cell phenotype, which may be of benefit in the treatment of peripheral nerve injuries and for promoting neurite outgrowth in vitro.[14] We have previously reported the beneficial effects of a cultured uncharacterized omental adipose-derived stromal vascular fraction.[15] However, to the best of our knowledge, there is little information in the literature concerning the effects of the combination of an artery graft and characterized uncultured adipose-derived nucleated cell fractions (ADNCs) on peripheral nerve regeneration in vivo in rats.

The aim of this study was to investigate the local effects of undifferentiated uncultured ADNCs combined with artery grafting on peripheral nerve regeneration. The present study was designed to determine if local ADNCs combined with artery grafting does in fact reduce dysfunction after a small gap nerve transection injury in the rat sciatic nerve transection model. The assessment of nerve regeneration was based on behavioural, functional (walking track analysis), muscle mass measurement, histomorphometric, and immunohistochemical (Schwann cell detection by S100 expression) criteria at 4, 8, and 12 weeks after surgery.

Materials and methods

Experimental design

Sixty male white Wistar rats weighing approximately 290 g were divided into four experimental groups ($n = 15$), randomly: (1) sham-surgery group (SHAM), (2) transected group (TC), (3) control group (Artery), and (2) ADNCs group (Artery/ADNCs). Each group was further subdivided into three subgroups of five animals each. Four donors were also assigned to ADNCs isolation and preparation. Twenty rats were used as artery graft donors, with two grafts taken from each rat. For 2 weeks before the experiment and during the entire course of the experiment, the animals were housed in individual plastic cages with an ambient temperature of 23 ± 3 °C, stable air humidity, and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. The rats underwent grafting procedures 3 days after the induction of diabetes.

Collection of adipose tissue and isolation of ADNCs

The entire abdomen was prepared aseptically, and after ventral midline incision, approximately 4–5 g omentum was harvested from each donor animal. The donor animals were then euthanized by anaesthetic overdose. The technique of ADNCs isolation has been described elsewhere.[15,16] In brief, the harvested omentum was rinsed with Hanks' buffered saline (HBS), trimmed, minced with two scalpels into very small pieces, and aspirated into a 10-ml pipette. The tissue was then transferred into a 50-ml Erlenmeyer flask containing 1500 U/ml collagenase type II (Sigma Chemical Co.). The ratio was 1 g of omental tissue to 2 ml of collagenase. The suspension of omental tissue and collagenase was incubated for 40 min in a 37 °C water bath with 100 shaking motions per min. The digested tissue was homogenized by repeated pipetting, transferred into a 15-ml tube, and centrifuged twice at $100 \times g$ for 5 min. The supernatant contained mainly adipocytes and the collagenase solution. The cell pellet was resuspended in 10 ml phosphate-buffered saline (PBS), filtered through a 150-μm pore-size mesh to remove non-digested large tissue fragments, and then washed two times with HBS. The ADNCs pellet was resuspended in sterile PBS solution in 10-μl aliquots (2×10^7 cells/ml), each loaded into a sterile syringe. The syringes containing PBS solution and ADNCs were shipped under chilled conditions to the investigators for immediate injection.

Grafting procedure and transplantation of ADNCs

Animals were anaesthetized by intraperitoneal administration of ketamine–xylazine (ketamine 5%, 90 mg/kg, and xylazine 2%, 5 mg/kg). The procedures were carried out in accordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain.[17] The university research council approved all experiments.

In the sham-operation group (SHAM), following surgical preparation, the left sciatic nerve was exposed through a gluteal muscle incision; after careful homeostasis, the muscle was sutured with resorbable 4/0 sutures and the skin with 3/0 nylon. In the TC group, the left sciatic nerve was exposed through a gluteal muscle incision and transected proximal to the tibio-peroneal bifurcation, where a 7-mm segment was excised, leaving a gap of approximately 10 mm due to retraction of the nerve ends. The proximal and distal stumps were each sutured to adjacent muscles. No graft was interposed between the stumps. In the Artery group, after transection and excision of 7 mm of the nerve, both the proximal and distal stumps were inserted 2 mm into an artery of 2 mm in diameter and 14 mm in length. Artery grafts were harvested from the abdominal aorta of the donor animals. The abdominal aorta artery was exposed