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Original article

Stromal vascular fraction combined with silicone rubber chamber improves sciatic nerve regeneration in diabetes

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ABSTRACT

Purpose: To study the effects of transplantation of characterized uncultured stromal vascular fraction (SVF) on sciatic nerve regeneration.
Methods: A 10-mm sciatic nerve defect was bridged using a silicone conduit filled with SVF. In control group, silicone conduit was filled with phosphate-buffered saline alone. In sham-operated group, the sciatic nerve was only exposed and manipulated. The regenerated nerve fibers were studied 8 and 12 weeks after surgery.
Results: Behavioral and functional studies confirmed faster recovery of regenerated axons in SVF transplanted animals than in control group ($p < 0.05$). Gastrocnemius muscle mass in SVF transplanted animal was found to be significantly more than that in control group. Morphometric indices of the regenerated fibers showed the number and diameter of the myelinated fibers to be significantly higher in SVF transplanted animals than in control group. In immunohistochemistry, the location of reactions to S-100 in SVF transplanted animals was clearly more positive than that in control group.
Conclusion: SVF transplantation combined with silicone conduit could be considered as a readily accessible source of stromal cells that improves functional recovery of sciatic nerve. It may have clinical implications for the surgical management of acute diabetic patients after facial nerve transection.

1. Introduction

Functional recovery following peripheral nerve injury remains a major challenge and functional recovery to preinjury level rarely occurs.[1] Recent therapeutic advances in the control of diabetes mellitus and diabetic neuropathy[2] have renewed the interest in the rate and quality of nerve regeneration in this chronic disease. Although measurable improvements may follow better control of blood sugar and administration of aldose reductase inhibitors, complete recovery is dependent on the regeneration of damaged axons and the reestablishment of fully functional connection with their targets.[3] To achieve maximum functional recovery, various techniques are being used. Employment of regenerative properties of stem cells at the service of nerve repair has been initiated during recent decades.[4] A widely accepted method by most surgeons is bridging the defect with an autologous donor nerve. Different graft equivalents have also been applied to bridge the nerve stump and regulated through the interaction of a variety of protein and cell signals.[5] It has been reported that using silicone tubes in bridging nerve defects could be promising because it is inert and does not induce extensive scarring or degeneration after implantation.[6] The advantages like no donor morbidity, availability, affordability and no foreign reactions make silicone rubber chamber an attractive alternative to other standard grafts.[7] It has been demonstrated that silicone rubber tubes are well tolerated in humans even after 3 years of implantation.[8] Silicone chambers are used as a standard experimental model to study the nerve regeneration process.[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

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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 identified that adipose tissue possesses a population of multi-potent stem cells which can be differentiated to a Schwann cell phenotype and may be of benefit for treatment of peripheral nerve injuries and promoting neurite outgrowth *in vitro*.[14] It has also been reported that differentiated adipose-derived cells enhance peripheral nerve regeneration.[15] Beneficial effects of cultured uncharacterized omental adipose derived stromal vascular fraction have already been reported.[16]

However, to the best of our knowledge, the literature concerning effects of combination of silicone conduit and characterized uncultured SVF on peripheral nerve regeneration *in vivo* in diabetic rats is rare. The objective of this study reported here was to evaluate effectiveness of characterized uncultured SVF as a readily accessible source of stromal cells on peripheral nerve regeneration using a diabetic rat sciatic nerve transection model. To achieve this, a silicone conduit was filled with uncultured SVF. Assessment of the nerve regeneration was based on behavioral and functional (walking track analysis) muscle mass measurement, and histomorphometric and immunohistochemical (Schwann cell detection by S100 expression) criteria 4, 8 and 12 weeks after surgery.

2. Materials and methods

2.1. Experimental design

Sixty male diabetic white Wistar rats weighing approximately 300 g were divided into four experimental groups ($n = 18$), randomly: Sham-operated group (SHAM), transected group (TC), control group (SIL) and SVF group (SIL/SVF). Each group was further subdivided into three subgroups with five animals in each. Four donors were also assigned to SVF isolation and preparation. Two weeks before and during the entire experiment, the animals were housed in individual plastic cages at an ambient temperature of 23° C ± 3° C, with stable air humidity, and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. For insulin-deficient diabetes, rats were fasted overnight before receiving a single intraperitoneal injection (50 mg/kg in 0.9% sterile saline) of streptozotocin (STZ). Hyperglycemia (≥15 mmol/L) was confirmed 2 days later by measurement of tail-vein blood glucose concentration. The rats underwent grafting procedures three days after induction of diabetes.

2.2. Collection of omental adipose tissue, isolation of SVF

The entire abdomen was prepared aseptically and after ventral midline incision approximately 4–5 g omentum were harvested from donor animals. The donor animals were then euthanized by over dose of the anesthetics. The technique of SVF isolation has been described elsewhere.[16,17] 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; and then the tissue was transferred into a 50-ml Erlenmayer flask containing 1500 U/ml collagenase type II (Sigma Co., USA). 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 at 100 shaking motions per minute. The digested tissue was homogenized by repetitive pipeting, transferred into a 15-ml tube, and centrifuged twice at 100 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-μ pore-size mesh to remove non-digested large tissue fragments, and then washed two times with HBS. The SFV pellet was resuspended in sterile PBS solution as 10-μL aliquots ($2–10^7$ cells/ml), each loaded into sterile syringes. The syringes containing PBS solution and SVF were shipped chilled to the investigators for immediate injection.

2.3. Grafting procedure and transplantation of SVF

Animals were anesthetized by intraperitoneal administration of ketamine–xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[18] The University Research Council approved all experiments.

Following surgical preparation in the SHAM group, the left sciatic nerve was exposed through a gluteal muscle incision and 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 tibioperoneal bifurcation where a 7 mm segment was excised, leaving a gap of about 10 mm due to retraction of nerve ends. Proximal and distal stumps were each sutured to adjacent muscles. In SIL group after transection and excision of 7 mm of the nerve, both proximal and distal stumps were inserted 2 mm into a silicone conduit, 2 mm in diameter and 14 mm in length, and two 10/0 nylon sutures were placed at each end of the cuff to fix the graft in place and to leave a 10-mm gap between the stumps. The conduit was filled with 10 μL phosphate buffered saline solution and sterile Vaseline was used to seal the ends of the tubes to avoid leakage. In the SIL/SVF group the conduit was filled with 10 μl aliquots ($2–10^7$ cells/ml) of SVF.

The animals were anesthetized (see above) and euthanized with transcardial perfusion of a fixative containing 2% paraformaldehyde and 1% glutaraldehyde buffer (pH = 7.4) at 4 ($n = 5$), 8 ($n = 5$) and 12 weeks ($n = 5$) after surgery.

2.4. Behavioral test

Functional recovery of the nerve was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale for rat hind limb motor function.[19] Although BBB is widely used to assess functional recovery in spinal cord injured animals, it has been demonstrated to be the most useful in assessment of never repair processes in peripheral nerve injuries.[20] Scores of 0 and 21 were given when there were no spontaneous movement and normal movement, respectively. A score of 14 shows full weight support and complete limb coordination. BBB recordings were performed by a trained observer who was blinded to the experimental design. The testing was performed in a serene environment. The animals were observed and assessed within a course of a 4-min exposure to an open area of a mental circular enclosure. BBB scores were recorded once before surgery in order to establish a baseline control and again weekly thereafter to assess functional recovery during 16 weeks.

2.5. Functional assessment of reinnervation

2.5.1. Sciatic functional index (SFI)

Walking track analysis was performed 4, 8 and 12 weeks after surgery based on the method of others.[20] The lengths of the third toe to its heel (PL), the first to the fifth toe (TS), and the second toe to the fourth toe (IT) were measured on the experimental side (E) and the contralateral normal side (N) in each rat. The sciatic function index (SFI) of each animal was calculated by the following formula: