The mesenchymal stem cell–derived microvesicles enhance sciatic nerve regeneration in rat: A novel approach in peripheral nerve cell therapy

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BACKGROUND: The accomplishment for desired functional peripheral nerve regeneration is still challenging despite various materials and methods. The effects of local application of omental adipose mesenchymal stromal cell–derived microvesicles (MVs) on peripheral nerve regeneration were studied using a rat sciatic nerve transection model.

METHODS: A 10-mm gap of sciatic nerve was bridged with a chitosan conduit. The rats were divided into five experimental groups randomly as follows: cultured undifferentiated omental adipose-derived stromal cells, rest mesenchymal stem cell–derived MVs (c-MVs), anti-inflammatory mesenchymal stem cell–derived MVs (anti-MVs), proinflammatory mesenchymal stem cell–derived MVs (pro-MVs), and negative control (Chit).

RESULTS: The functional assessment of nerve regeneration (walking track analyses), electrophysiologic measurements, muscle mass measurements, as well as histomorphometrical and immunohistochemical indices showed drastic improvement in nerve regeneration in c-MVs and anti-MVs animals compared with pro-MVs animals (p < 0.05).

CONCLUSION: The anti-inflammatory stem cell–derived MVs can be used as an alternative for the improvement of rat sciatic nerve regeneration. (J Trauma Acute Care Surg. 2014;76: 991–997. Copyright © 2014 by Lippincott Williams & Wilkins)

KEY WORDS: Stem cell–derived microvesicles; sciatic nerve regeneration; omental adipose-derived stromal cells; rat.

Peripheral nerve injuries are common in clinical practice owing to trauma or deliberate surgical resection.1 Autologous nerve graft is widely accepted as the most effective procedure for repairing a neural gap; however, the availability of donor nerves and donor site morbidity are major concerns.2 Therefore, numerous surgical methods such as bioabsorbable or nonbioabsorbable conduits are being used for bridging nerve defects.3–5 It has been approved that cellular elements are needed to provide the neurotropic and neurotrophic support for axonal regrowth.5

Cell transplantation, autologous Schwann cells, and stem cell therapy have been successfully used for the improvement of peripheral nerve regeneration.6 The benefit of vascular fraction7 and cultured or uncultured undifferentiated multipotent mesenchymal stem cells (MSCs) for the treatment of peripheral nerve injuries have been identified.8–11

Microvesicles (MVs) are nano-sized circular membrane fragments that are produced from cells and act as shuttles for selective pattern of ligands, receptors, enzymes, cytokines, transcription factors, messenger RNA, and microRNA into target cells. After the attachment of MVs into the target cells or their internalization, various epigenetic reprogramming and phenotypic changes ensue.12–14 Furthermore, MSC-derived MVs mostly bear receptor repertoire and donor ligand cells.15

Recent studies have shown that MSC-derived MVs contribute in the treatment of the injured organs or tissues.16–18 Therapeutic application of MSCs bears some limitations.17–21 The objective of the present study was to evaluate effectiveness of local application of omental adipose mesenchymal stromal cells (s)–derived MVs on peripheral nerve regeneration in a rat sciatic nerve transection model.

MATERIALS AND METHODS

Experimental Design

Ninety male White Wistar rats weighing approximately 220 g were randomly divided into six groups (n = 15), including the sham operation group (sham) and five treatment groups as follows: negative control conduit group (Chit), cultured undifferentiated omental adipose-derived stromal cells group (OADSCs group as a positive control), rest MSC-derived MVs (c-MVs) group, anti-inflammatory MSC-derived MVs (anti-MVs) group, and proinflammatory MSC-derived MVs (pro-MVs) group. Each group was further subdivided into three subgroups of five animals each.

The animals were treated under the standard condition during entire experiment. All procedures were carried out in accordance with the guidelines of the ethics committee,22 and the University Research Council approved all experiments.

Submitted: October 9, 2013, Revised: November 29, 2013. Accepted: December 26, 2013.

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DOI: 10.1097/TA.0000000000000186

J Trauma Acute Care Surg
Volume 76, Number 4
**TLR Priming Protocol**

Lipopolysaccharide (10 ng/mL; Sigma-Aldrich, St. Louis, MO) and poly IC (1 μg/mL; Sigma-Aldrich) were added to OADSCs culture growth medium at 60% to 70% confluency as agonists for TLR4 and TLR3, respectively. After 1-hour incubation at 37°C and 5% CO₂ atmosphere, growth medium was refreshed without the addition of TLR agonists, and MVs were isolated from conditioned medium during next 12 hours. Short incubation and minimal concentration of TLR agonists exposure were used according to Waterman et al., to mimic the gradient of danger signals.

**Isolation of MVs**

Isolation of MVs was based on a modification protocol of Thery et al. (2006). Briefly, 12 hours after TLR priming, collected OADSCs culture supernatant at second passage were centrifuged at 2,000 G for 20 minutes and 100,000 G for 1 hour. The resultant pellet was suspended in an acidic buffer (140-mM NaCl, 10-mM citrate, pH 4) to remove residual receptor-bound agonists. After 60 seconds, phosphate-buffered saline (PBS) was added to MVs suspension and then was centrifuged again at 100,000 G for 1 hour. The final MVs pellet was suspended in PBS. Bradford assay was used to quantify protein content of resultant MVs.

**Electron Microscopy**

Ten microliters of MV suspension (10 μg/100 μL) was loaded on a formvar-coated copper grid. Negative staining was performed by the addition of 10 μL of neutral 1% aqueous phosphotungestic acid. The grids were examined under transmission electron microscope (Philips Bio Twin, CM100, the Netherlands) at 75 kV, and electromicrographs were taken and subjected to analyses. Analyses of MVs by electron microscope showed the presence of nano-sized, MVs (Fig. 1).

**Grafting Procedure**

Animals were anesthetized using ketamine hydrochloride 90 mg/kg (Ketaset 5%, Alfasan, Woerden, the Netherlands) and xylazine hydrochloride 5 mg/kg (Rompun 2%, Bayer, Leverkusen, Germany) intraperitoneally. In the sham group, the left sciatic nerve was exposed through a gluteal muscle splitting, and following exposure of sciatic nerve, the splitted muscle was closed using 4/0 Vicryl (Ethicon, Norderstedt), and the skin using 3/0 nylon (Dafilon, B/Braun, Germany). In the treatment groups, the left sciatic nerve was exposed as mentioned before and transected proximal to the tibioperoneal bifurcation. An 8-mm segment was excised, and a gap approximately 10 mm was made because of the retraction of the nerve ends. The created stumps were each inserted 2 mm into a 14-mm chitosan conduit, and two 10/0 nylon sutures were placed at each end to fix the conduit in place. In the treatment groups, the conduit was filled with 1 × 10⁵ OADSCs, 45-μg c-MVs, 45-μg anti-MVs, and 45-μg pro-MVs, respectively, all prepared up to 30 μL with PBS solution, accordingly. In the negative control group, the conduit was filled with 30-μL PBS solution.

The collection of omental adipose tissue and preparation of cultured undifferentiated omental adipose-derived stromal cells were based on techniques described in our pervious study. The efficacy of the conduit on peripheral nerve regeneration in a rat model has been described in other previous study.

At the end of the experiment, animals were anesthetized as mentioned before and were perfused via the left cardiac ventricle with a fixative containing 2% paraformaldehyde and 1% glutaraldehyde buffer (pH 7.4) at 4, 8, and 12 weeks after surgery.

**Functional Assessment of Nerve Regeneration**

We adopted a walking track analysis at 4, 8, and 12 weeks after surgery based on a method described by others. In brief, the lengths of the third toe to its heel (PL), the first to the fifth toe (IT) were measured on the operated side (E) and the contralateral unoperated side (N) in each rat. The sciatic function index (SFI) in each animal was calculated using the following formula:

\[
SFI = -38.3 \times \frac{(EPL - NPL)}{NPL} + 109.5 \times \frac{(ETS - NT)}{NTS} + 13.3 \times \frac{(EIT - NIT)}{NIT} - 8.8
\]

Overall, the SFI approximately 0 was considered for normal nerve function and around −100 SFI for total dysfunction. The SFI was assessed based on the sham group, and the normal level was considered as 0.

**Electrophysiologic Measurement**

After 12 weeks, the animals were subjected to electrophysiologic studies using Nacro bio system 320-3760 A trace 80. Under general anesthesia (discussed earlier), left sciatic nerve was reexposed. Single electrical pulses (at supramaximal intensity) were delivered via bipolar electrodes placed in turn at the proximal and distal trunk of the regenerated nerve cable and electromyography (EMG) was recorded by inserting an electrode into the belly of gastrocnemius muscle. The latency and amplitude of EMG were obtained. Difference in latency of EMG