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Short communication

Intramuscular injection of bone marrow mesenchymal stem cells with small gap neurorrhaphy for peripheral nerve repair

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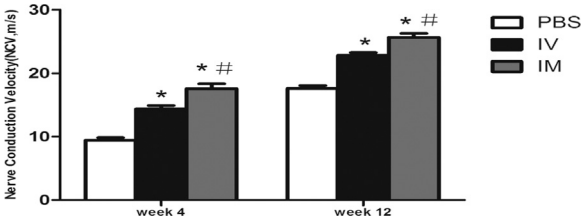
HIGHLIGHTS

- We have proposed a novel small gap neurorrhaphy for peripheral nerve mutilation.
- Effects of BMSCs injected intramuscularly compare with that injected intravenously.
- Intramuscular injection of BMSCs could promote peripheral nerve regeneration.
- Intramuscular injection of BMSCs could delay denervated muscle atrophy.

GRAPHICAL ABSTRACT



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ABSTRACT

We had previously reported that small gap neurorrhaphy by scissoring and sleeve-jointing epineurium could enhance the rate and quality of peripheral nerve regeneration. To date, local implantation and systemic delivery of bone marrow mesenchymal stem cells (BMSCs) have been routinely used in nerve tissue engineering, but they each have some intrinsic limitations. We hypothesised that targeted muscular administration of BMSCs capable of reaching the damaged nerve would be advisable. Here, we investigated the therapeutic efficacy of transplantation of BMSCs through targeted muscular injection with small gap neurorrhaphy by scissoring and sleeve-jointing epineurium on repairing peripheral nerve injury in a rat model. One week after a rat model of peripheral nerve injury was established by small gap neurorrhaphy, thirty-six Sprague–Dawley rats were randomly divided into three groups ($n = 12$): the intramuscular injection of BMSCs group (IM), the intravenous injection of BMSCs group (IV) and the intramuscular injection of phosphate-buffered solution group (PBS). The process of the nerve regeneration was assayed functionally and morphologically. The results indicated that compared to the IV-treated and PBS-treated groups, the targeted muscular injection therapy resulted in much more beneficial effects, as evidenced by increases in the sciatic function index, nerve conduction velocity, myelin sheath thickness and restoration rate of gastrocnemius muscle wet weight. In conclusion, the combination therapy of small gap neurorrhaphy and BMSC transplantation through targeted muscular injection can significantly promote the regeneration of peripheral nerve and improve the nerve's functional recovery, which may help establish a reliable approach for repairing peripheral nerve injury.

1. Introduction

Epineurium or perineurium end-to-end neurorrhaphy to recover nerve continuity has been the most commonly used repair method for peripheral nerve mutilation. However, the functional

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recovery is unsatisfactory in many cases [1]. Since the nerve selective regeneration theory was proposed, many researchers have focused on using autologous tissue and biodegradable conduits to construct a nerve regeneration chamber [2,3]. Autologous materials such as artery, vein or fascia have limitations, including graft resources, requirement of a donor site located distant from the original surgical site and insufficient length or diameter to optimise the repair [4]. Although synthetic conduits have overcome the aforementioned problems, their clinical application is limited by the high expense and possibly foreign bodies to the host [5].

To improve the functional outcome and reduce complications of peripheral nerve repairs, we have proposed small gap neurorrhaphy of scissoring and sleeve-jointing autologous epineurium to construct a nerve regeneration chamber for the repair of peripheral nerve mutilation. Our previous study demonstrated that this method could improve the nerve regeneration effect and could be an effective alternative approach for the repair of peripheral nerve mutilation [6].

Previous studies have demonstrated that bone marrow mesenchymal stem cells (BMSCs) can differentiate into Schwann-like cells and support nerve regeneration after their transplantation into different models of peripheral nerve injury [7,8]. Although BMSCs have the ability to specifically root in a damaged area, the number of BMSCs detected in these tissues is exceedingly low [9,10]. Therefore, efficient delivery of BMSCs to sites of intended action is necessary.

Currently, stem cell transplantation in the treatment of peripheral nerve injury is performed using local and intravenous injection. However, both methods have some limitations [11–13]. Research has shown that exogenous neurotropic factors via targeted muscular injection in a muscle atrophy amyotrophic lateral sclerosis rat model could be expressed in spinal cord anterior horn cells and prolong the rats' survival time [14–16]. Therefore, we surmised that transplantation of BMSCs via targeted muscular injection could promote nerve regeneration and prevent denervated muscle atrophy. The aim of the current study was to evaluate the therapeutic efficacy of the transplantation of BMSCs through intramuscular injection combined with small gap neurorrhaphy by scissoring and sleeve-jointing epineurium for peripheral nerve mutilation in a rat model.

2. Materials and methods

2.1. Animals

Forty-six male Sprague–Dawley (SD) rats weighing 200–250 g were provided by the experimental animal centre of Soochow University, China [Certification No. SYXK (Su) 2007-0035]. All experimental animals were housed under standard conditions. Animals were anesthetised by intraperitoneal injection of chloral hydrate (350 mg/kg weight) during the surgical procedure. All protocols were reviewed and approved by the institutional laboratory animal care and use committee, and carried out in accordance with the National Institutes of Health guide for the care and use of animals for experimental procedures. All efforts were made to minimise both the number and suffering of the animals used.

2.2. Isolation and culture of BMSCs

Ten SD rats were sacrificed with a lethal dose of chloral hydrate. The BMSCs were isolated from bilateral femurs as previously described [17] and cultured in low-glucose Dulbecco's Modified Eagle's Medium (L-DMEM, Gibco, USA) containing 10% foetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin at 95% humidity with 5% CO_2 at 37 °C. BMSCs at passage 3 were used for further experiments. The morphology of BMSCs at each passage was examined under inverted phase contrast microscopy (IMT-2, Olympus, Japan).

2.3. Flow cytometry analysis of BMSCs

Flow cytometry analysis was performed to characterise the phenotypes of cultured BMSCs. Briefly, BMSCs at passage 3 were washed twice with phosphate-buffered solution (PBS; Gibco, USA), then trypsinised with 0.25% trypsin (Sigma, USA) into cell suspension, centrifuged for 5 min at 800 rpm, and adjusted to a cell density of approximately 1×10^6 cells/ml. Then, 100 μl of the cell suspension was incubated with monoclonal antibodies against mouse CD29, CD34, CD45, CD90 (All BD Biosciences, USA) overnight at 4 °C. The cells were washed twice in PBS and incubated with anti-rat antibody CD29-FITC, CD34-PE, CD45-AlexaFluor647 and CD90-PerCP/Cy5.5 (all from BD Biosciences, USA) as the secondary antibody at room temperature for 60 min. Flow cytometry analysis was performed after two final PBS washes using a FACSCalibur flow cytometry apparatus coupled with Cell Quest Pro Software (BD Biosciences, USA).

2.4. Surgical procedure

The rat model of small gap neurorrhaphy to repair peripheral nerve rupture using a nerve regeneration chamber was constructed by scissoring and sleeve-jointing autologous epineurium as we previously described [6]. Thirty-six male SD rats were used. Briefly, after the anaesthesia took effect, the left sciatic nerve was exposed from the sciatic notch to its bifurcation into the tibial and peroneal nerves. The sciatic nerve was transected at 10 mm above the sciatic nerve bifurcation. In the proximal stump, a 1-mm-long epineurium annular section was dissociated and removed. In the distal stump, a 3-mm-long epineurium segment was longitudinally incised. We then dissected the epineurium to separate the epineurium and nerve bundle with scissors. After the epineurium was dissociated from proximal to distal, the nerve bundle was cut off at the summit of the longitudinal incision. After pulling the distal epineurium, we created a sleeve jointing with the proximal nerve epineurium with a 2 mm gap between the two nerve stumps. The epineurium of the two stumps was sutured at the site without tension 1 mm away from the proximal nerve stump with 10-0 stitches (Prolene, Ethicon, USA). The longitudinal dissected epineurium was then sutured, followed by the muscular layer and the skin.

2.5. Groups and transplantation procedure

One week after neurorrhaphy, BMSCs used for qualitative (immunohistochemical) studies were labelled with 5-bromo-2-deoxyuridine (BrdU, Abcam, UK) 48 h prior to transplantation. Approximately, 1×10^6 BMSCs in 1 ml total fluid volume of PBS were prepared for the study. The 36 rats were randomly divided into three groups ($n = 12$): group I (IM group): 1 ml PBS and BMSCs suspensions were injected into the medial and lateral heads of the gastrocnemius muscle; group II (IV group): 1 ml PBS and BMSCs suspensions were injected into the tail vein; and group III (PBS group): 1 ml PBS was injected into the medial and lateral heads of the gastrocnemius muscle.

2.6. Functional assessment

An investigator who was blinded to the experimental allocation evaluated the sciatic function index (SFI) at weeks 4, 8 and 12 post-neurorrhaphy. Briefly, 6 rats in each group were randomly selected, the plantar surface of the hind feet was dipped into black ink, and the rats were allowed to ambulate down a wooden tunnel so that