Bone Marrow Mesenchymal Stem Cell and Vein Conduit on Sciatic Nerve Repair in Rats

Kamal Seyed Foroutan 1,*, Ali Khodarahmi 2, Hootan Alavi 1, Sepehr Pedram 3, Mohamad Reza Baghaban Eslaminejad 4; Sima Bordbar 4

1Department of Plastic Surgery, Hazrat Fatemeh Hospital, Iran University of Medical Sciences, Tehran, IR Iran
2Department of Plastic Surgery, Bahonar Hospital, Kerman University of Medical Sciences, Kerman, IR Iran
3Veterinary Faculty, Hazrat Fatemeh Hospital, Iran University of Medical Sciences, Tehran, IR Iran
4Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, IR Iran

*Corresponding author: Kamal Seyed Foroutan, Department of Plastic Surgery, Hazrat Fatemeh Hospital, Iran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2188890889, E-mail: k_sforootan@yahoo.com

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Background: Peripheral nerve repair with sufficient functional recovery is an important issue in reconstructive surgery. Stem cells have attracted extensive research interest in recent years.

Objectives: The purpose of this study was to compare the vein conduit technique, with and without the addition of mesenchymal stem cells in gap-less nerve injury repair in rats.

Materials and Methods: In this study, 36 Wistar rats were randomly allocated to three groups: In the first group, nerve repair was performed with simple neurorrhaphy (control group), in the second group, nerve repair was done with vein conduit over site (vein conduit group) and in the third group, bone marrow stem cells were instilled into the vein conduit (stem cell group) after nerve repair with vein conduit over site. Six weeks after the intervention, the sciatic function index, electrophysiological study and histological examination were performed.

Results: All animals tolerated the surgical procedures and survived well. The sciatic function index and latency were significantly improved in the vein conduit (P = 0.04 and 0.03, respectively) and stem cell group (P = 0.02 and 0.03, respectively) compared with the control group. No significant difference was observed in sciatic function and latency between the vein conduit and stem-cell groups. Moreover, histological analysis showed no significant difference in regenerative density between these two groups.

Conclusions: The results of this study showed that the meticulous microsurgical nerve repair, which was performed using the vein tubulization induced significantly better sciatic nerve regeneration. However, the addition of bone marrow mesenchymal stem cell to vein conduit failed to promote any significant changes in regeneration outcome.

Keywords: Bone Marrow; Mesenchymal Stromal Cells; Regeneration; Trauma

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3. Materials and Methods

3.1. Study Design and Animals

In this study, 36 Wistar rats (3-4 months old), weighting between 300 and 350 g were used. They were housed in cages, and maintained on a 12-hour light-dark cycle with free access to water and food. The rats were randomly allocated to three groups (n = 12, each group); in the first group nerve repair was performed with simple neurorrhaphy (group A: control group), in the second group nerve repair was done with vein conduit over neurorrhaphy (group B: vein conduit group) and in the third group after nerve repair with vein conduit over neurorrhaphy, marrow stem cells were instilled into the vein conduit (stem cell group). The animals were anesthetized with ketamine (90 mg/kg) and xylazine (9 mg/kg) and Ketamine was repeated if necessary during the procedure. Sciatic nerve function was evaluated for all groups at the beginning of the study; then, stem cell isolation and culture were performed in group C. Thereafter, an appropriate surgical intervention was done. Six weeks after the intervention, the sciatic function evaluation was repeated, electrophysiological study and histological examinations were also performed prior to euthanasia.

3.2. Bone Marrow Cell (Mesenchymal Stem Cells) Harvest

Under general anesthesia, 0.4 mL bone marrow were aspirated from left tibia of rat using 21 gauge needle, and mixed with 5 mL Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 IU/mL streptomycin. Bone marrow stem cells were washed by centrifugation for 3 minutes at 1200 rpm and followed by discarding the supernatant. The pellet was suspended in 1 mL DMEM and plated in 75 cm² culture flasks at density of 10³ cells per mL in a 15 mL DMEM containing 15% FBS and antibiotics. The cultures were incubated in an atmosphere of 5% CO₂ at 37°C. Three days after culture initiation, the medium was changed to discard the nonadherent cells. The cultures were then allowed to achieve 70-80% confluency. At this time, they were trypsined and subcultured at 1:3 ratio. Two additional passages were performed to obtain sufficient cells, which were used to conduct the following experiments and cells were used after 3 passage and mesenchymal stem cell confirmation (surface markers, bone, cartilage and adipose differentiation).

3.3. Surgical Procedures

After the animals were anesthetized, the right sciatic nerve was exposed through an incision over the gluteal muscle incision and a 2-3 mm segment of the nerve was excised above the bifurcation area. The nerve specimen was sent for histological examination. In all groups, neurorrhaphy was performed under × 4.0 magnification loupe with two or three 11-0 nylon epineural sutures. For nerve conduit group a segment of right-sided jugular vein with an average length of 2 cm was harvested from right side and then proximal end of transected sciatic nerve was passed through the vein and after neurorrhaphy vein segment rolled to cover the repair site. For the stem cell group, repair was fashioned in the same way as the nerve conduit group, but after the completion of procedure 250 ×10³ previously prepared cells suspended in 0.2 mL fibrin glue were injected into the vein. After completion of the procedure, skin was repaired with 4-0 nylon suture (Figure 1).

Figure 1. Surgical Procedure; (A-D): after exploration, a segment of vein is excised on to a tube; thereafter, vein segment is transferred on to a device to facilitate its use as a conduit; (E) final result after neurorrhaphy and its rolled vein segment to cover the repair site.
Table 1. Data Analysis for Each Experimental Group a, b, c

| Groups b | Axon Count /HPF | Postop Electrophysiology | Sciatic Function Index |
|----------|-----------------|---------------------------|-----------------------|
|          | Preop           | Postop                    | Study: Latency mSec    | Preop        | Postop    |
| Group A (n = 12) | 176 ± 36       | 133 ± 34                  | 1.55 ± 0.21           | -9.2 ± 3.99 | -8.18 ± 5.06 |
| Group B (n = 12) | 170 ± 31       | 149 ± 26                  | 1.35 ± 0.21           | -10.1 ± 8.01 | -7.08 ± 12.96 |
| Group C (n = 12) | 163 ± 32       | 144 ± 29                  | 1.34 ± 0.12           | -14.27 ± 9.09 | -6.9 ± 11.9 |
| Significance (P values) | None | none | A vs. B: 0.038; A vs. C: 0.030 | None | A vs. B: 0.046; A vs. C: 0.028 |

a Abbreviations: Preop, preoperative period; Postop: postoperative period
b Group A: simple neurorrhaphy, group B: vein conduit over neurorrhaphy, group C: vein conduit containing bone marrow derived stem cells over neurorrhaphy.

3.4. Functional Evaluation

Functional aspect of sciatic nerve was expressed by sciatic nerve function index (SFI) using the method described by Reynolds and Weiss (12). Rat’s hind feet were dipped in ink and the rats were allowed to walk across a tunnel so that the footprints could be recorded on white paper loaded onto the bottom of the tunnel. The footprints of normal feet (N) and experimental feet (E) were measured and evaluated with 3 indices: length of the footprint from third toe to heel (PL), width of toes from 1st to the 5th toe (TS) and width of middle toes from 2nd to 4th toe (ITS). The SFI was calculated according to the formula described by Varejao et al. (13): SFI=-38.3 [(EPL-NPL)/(NPL)] + 109.5 [(EITS-NTS)/(NTS)] + 13.3 [(EITS-NTS)/(NTS)] - 8.8. SFI was calculated twice: once before an intervention for observing primary function of sciatic nerve and second, six weeks after operation for evaluation of regeneration quality. In general, SFI oscillates around 0 for normal nerve function, and around -100 for complete nerve dysfunction (14).

3.5. Electrophysiological and Histological Study

For electrophysiological and histological study the rats were anesthetized and sciatic nerves were re-exposed. Electrophysiological tests were performed by application of an electrical stimulation (duration of 0.1-0.2 milliseconds, frequency of 1 Hz, and intensity of 15 mm) to the proximal side of the injured sites, and a recording electrode in the extensor digitorum (14, 15). The distance between two electrodes was measured by a caliper. The onset latency was recorded. Then, nerve dissection was performed at and below the repair site. A block of nerve distal to the repair site was excised. After preparation of nerve specimens; evaluation with × 100 magnification (Olympus, PROVIS Ax 70, Japan) nerve axon count was done randomly for four separate fields and the average was reported as axon count.

3.6. Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis, after implementing Kolmogorov-Smirnov test for normality, was performed using one-way ANOVA and post-hoc tests with the SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). P < 0.05 was considered statistically significant.

4. Results

All animals tolerated surgical procedures and survived well without any serious surgical complication. Preoperative and postoperative results of sciatic function index, axon count and nerve conduction velocity (latency in milliseconds) are shown in Table 1 and Figure 2. After six weeks, gait analysis and electrophysiological study revealed better sciatic function index and latency in vein conduit and stem cell groups. The sciatic function was enhanced about 12% in the Vein conduit group (P = 0.04) and 14% in the stem-cell (P = 0.02) group compared to the control group. Also, latency was diminished about 13% in the vein conduit (P = 0.03) and 14% in the stem cell (P = 0.03) group compared with the control group.

Histological analysis, including mean axon count density was performed in all groups and compared to the normal proximal sciatic nerve blocks. In comparison to proxi-
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