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Motoric Recovery After Transplantation of Bone Marrow Derived Mesenchymal Stem Cells in Chronic Spinal Cord Injury: A Case Report

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Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Conflict of interest: None declared

Patient: Male, 35
Final Diagnosis: Chronic spinal cord injury
Symptoms: Paraplegia
Medication: —
Clinical Procedure: Bone marrow derived mesenchymal stem cell injection
Specialty: Orthopedics and Traumatology

Objective: Unusual or unexpected effect of treatment

Background: For the past 20 years, numerous of clinical trials focusing on the use of mesenchymal stem cells (MSC) in spinal cord injury (SCI) treatment has been conducted. However, controversies over whether stem cells are the main factor in a patient's recovery still persisted in sub-acute SCI. This study aimed to evaluate the motoric recovery in a chronic SCI patient treated with bone marrow derived MSC (BM-MSC) transplantation.

Case Report: We present a case report of patient with a 12-year-long-chronic SCI that was treated by BM-MSC) transplantation using a serial administration protocol. The protocol consisted of direct parenchymal injection to the affected lesion and multiple (5 times) intravenous stem cell injection as the adjuncts. There was no complication or serious adverse effects encountered during the procedure and follow up. At the final follow up of 5 years, the patient neurological status improved from American Spinal Injury Association (ASIA) A status to ASIA C status, which signifies improvement in his ambulatory status. Magnetic resonance imaging and electrophysiology examination also showed changes that indicated recovery of the neurologic function.

Conclusions: Based on the limited adverse reaction and outcome, our case report may serve as an additional alternative protocol in stem cell administration to improve the outcome of chronic spinal cord injury patients.

MeSH Keywords: Adult Stem Cells • Mesenchymal Stem Cell Transplantation • Spinal Cord Injuries

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/917624

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Background

Spinal cord injury (SCI) is considered a debilitating condition, with a limited chance for recovery. Severe disability and financial burden due to SCI are major concern not only for the patients, but also for the family, caretaker, and the community [1,2].

For the past 20 years, there have been numerous clinical trials evaluating the use of stem cells for SCI [3]. Several translational studies reported the success of stem cells in treating the SCI in the sub-acute phase. However, controversies over whether stem cells are the main factor in the patient's recovery still persisted. The success of stem cell treatment in chronic SCI cases might prove its potential, but the current limited results have discouraged enthusiasm for stem cells use in recent years [4,5].

In this case study, we performed a clinical trial using bone marrow derived mesenchymal stem cells (BM-MSCs) to treat a patient with chronic SCI, who had undergone numerous treatment methods with no avail. Improvement of the motoric function was the highlight in this case, which was not found in previous published trials.

Case Report

Initial clinical background

In 2014, a 35-year-old male was admitted to our institution with chronic SCI due to a gunshot in his T12 spine, 13 years before. At that time, he underwent immediate decompression and bullet extraction surgery. However, after the surgery and a series of physical therapies, there was no improvement in his motoric and sensory function, as he remained paraplegic (American Spinal Injury Association A status) for 13 years. He also complained of intermittent radiating pain from the lumbar area to both lower extremities. Bladder and bowel function were also totally disrupted after the initial injury, however slight improvements were observed over time. The patient was able to urinate voluntarily and felt the needs to urinate at the initial admission to our center.

On physical examination, there was diminished motoric and sensory below the level of T12. No pathological reflex or hyper-reflexes were found. Anal tone was diminished. Additional examinations were performed to determine the extent of damage and the remaining function of nerve/muscle groups. Nerve conduction study showed a bilateral total conduction block at the level of T12. Urodynamic study resulted in lower motor neuron type of neurogenic bladder with minimal trace of detrusor contraction during urination. The examination also couldn't detect any pelvic floor muscle contraction. Lumbosacral magnetic resonance imaging (MRI) revealed an old infarct of conus medullaris with transverse myelitis at the level of Th10–L1 (Figure 1).

The clinical trial was approved by the institutional board review (DM.03.01/II.3/1366/2014) and was conducted immediately in 2014. Written informed consent and the baseline status were obtained prior to the trial.

Isolation and culture of bone marrow derived mesenchymal stem cells (BM-MSCs)

Autologous bone marrow derived mesenchymal stem cells (BM-MSCs) were isolated and cultured based on protocol previously described by Lubis et al. [6]. Bone marrow harvest was performed under local anesthesia (lidocaine 2%) in an outpatient clinic. Sixty milliliters bone marrow was aspirated from several locations around the posterior iliac crest and was contained into 6 syringes, each prefilled with 2 mL of heparin (5000 U/mL). The aspirated bone marrow was then diluted with phosphate-buffered saline (PBS) on 1: 1 ratio and centrifuged in room temperature at 3000 rpm for 30 minutes. The collected buffy coat was washed and transferred into culture flask containing Dulbecco's Modified Eagle Medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). Cells were incubated in 37°C at 5% CO_2 with routine culture medium change every 2–3 days. Subculture was performed within day 9.

All cells were implanted on the third passage. The first implantation was conducted using fresh harvested cell on Day 33. All cells for intravenous boosters were cryopreserved on the second passage. Five intravenous boosters were conducted monthly for 5 consecutive month, using thawed cryopreserved cells, and then expanded for 5–6 days until the cells reached approximately 10×10^6 cells. Total harvested cells for 6 injections were 69.5×10^6 cells.

Cellular characterization was then performed on plastic adherent confluent cells by using flow cytometry (FACSCalibur™, Franklin Lakes, NJ, USA). Cultured cells were checked for typical MSCs markers (CD73, CD90, CD105) and hematopoietic markers expression (HLA-DR, CD14, CD19, CD34, and CD45). To ensure safety, sterility of the BM-MSCs was checked 3 times throughout the culture process. BM-MSCs culture expansion was conducted in a cGMP certified facility (ReGeniC Laboratory – Bifarma Adiluhung, Jakarta, Indonesia). Cell viability was evaluated with trypan blue staining.

BM-MSCs administration protocol

The BM-MSCs administration protocol consisted of 2 phases: 1) intradural injection of the BM-MSCs through mini open

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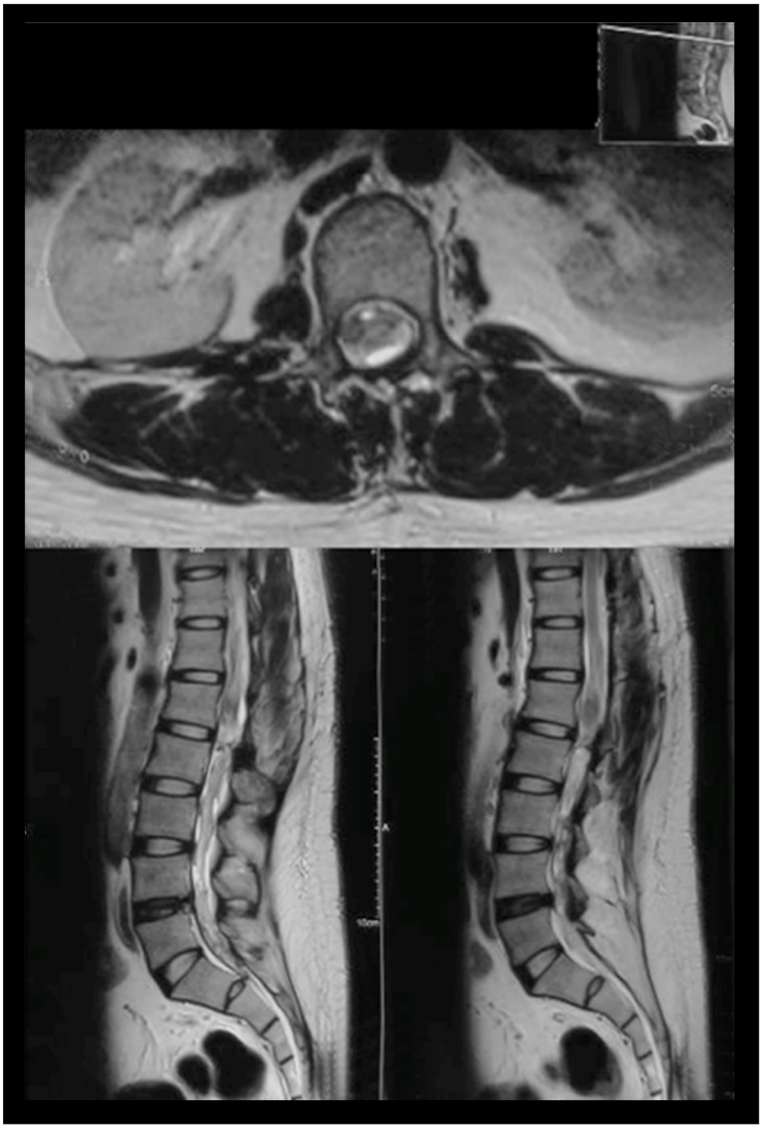

Figure 1. T2 weighted magnetic resonance imaging showing myelomalacia starting from Th12 with no/minimal compression of the spinal canal.

surgical approach; and 2) monthly intravenous BM-MSCs injection (for 6 months).

The first phase was performed by intradural injection of BM-MSCs in the operating theater under general anesthesia. Limited posterior approach of the spine was conducted and L1 laminotomy was performed exposing the injured spinal cord. A 23G needle was introduced to the injured area (conus medullaris between L1 and L2), then 3 mL serum containing 1.6×10^7 BM-MSCs were injected slowly. The syringe was left in place for 3 minutes to allow dilution of the cells into the damaged cord and prevent the immediate leakage of the cells through the injection site, before slowly being withdrawn. The surgical wound was closed in regular manner without any drain and the patient was observed for 3 days in the ward before being discharged.

One month later, the patient was readmitted for the first intravenous BM-MSCs injections. The patient's baseline vital signs were recorded before the injection. Around 1.7×10^7 BM-MSCs contained in 1 mL serum was diluted with 50 mL of normal saline (NaCl 0.9%, B. Braun, Melsungen, Germany) and was infused at the rate of 200 mL/hour. The patient's vital signs were monitored throughout the process and for the following 24 hours. No complication was found during the first injection. The remaining 5 injections were performed in a similar manner with 1-month interval between the injections. Each injection contained $1.0–1.2\times10^7$ third passage BM-MSCs.

No major complication was found during the serial intravenous injection processes. However, the patient sustained a hyperthermia on the second injection, which was treated successfully using antipyretic medication. On the third injection, there was a fleeting glimpse of malignant hypertension

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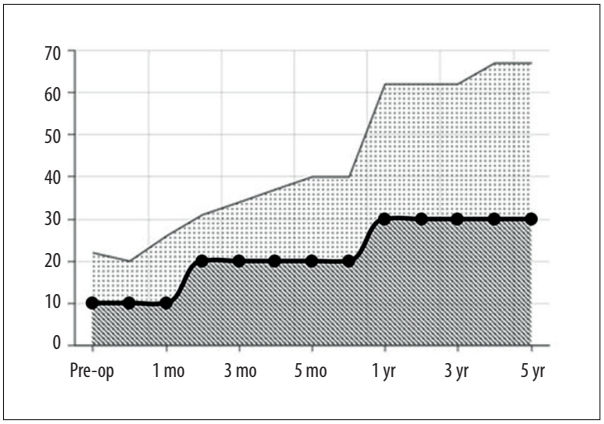

Figure 2. Progression of neurological improvement (ASIA Score – black dots) and improvement in physical and mental function evaluated using Short Form-36 questionnaire (grey line).

(200/140 mmHg), which was only observed since the blood pressure returned to baseline immediately and no abnormalities were detected in clinical examination.

The rehabilitation therapy was initiated after the first injection, consisting of functional recovery exercise and urinary retention training. Clinical neurological examination, functional outcome index (Oswerty Disability Index and Short-Form 36) were documented after each injection procedure, and annually until the final 5-year follow up. MRI examination was re-evaluated at the final follow up.

Result and Follow Up

During the first 6 months (during the serial injections), there was no significant improvement in functional scoring, or motoric and sensory function, although, subjectively, the patient felt minimal sensory improvement around his lower extremities.

During the mid-term follow up, there was progressive increase of the patient's motoric power (0 into 3), which occurred in the muscle groups that was innervated by the L1 and L2 nerve roots (hip flexors). This was shown by the patient's ability to crawl independently after 1 year of follow up (Supplementary Video 1). There was no increase of power in the other muscle group until the final follow up (5 years). Improvements of functional outcome are summarized in Figure 2.

MRI images at the final follow up showed minimal changes compared with the initial images. The transverse myelitis was slightly decreased meanwhile the old infarct remained (Figure 3).

Electromyography examination showed new fibrillations in L2 innervated muscles, which may suggest signs of re-innervations. Somatosensory evoked potential study showed no

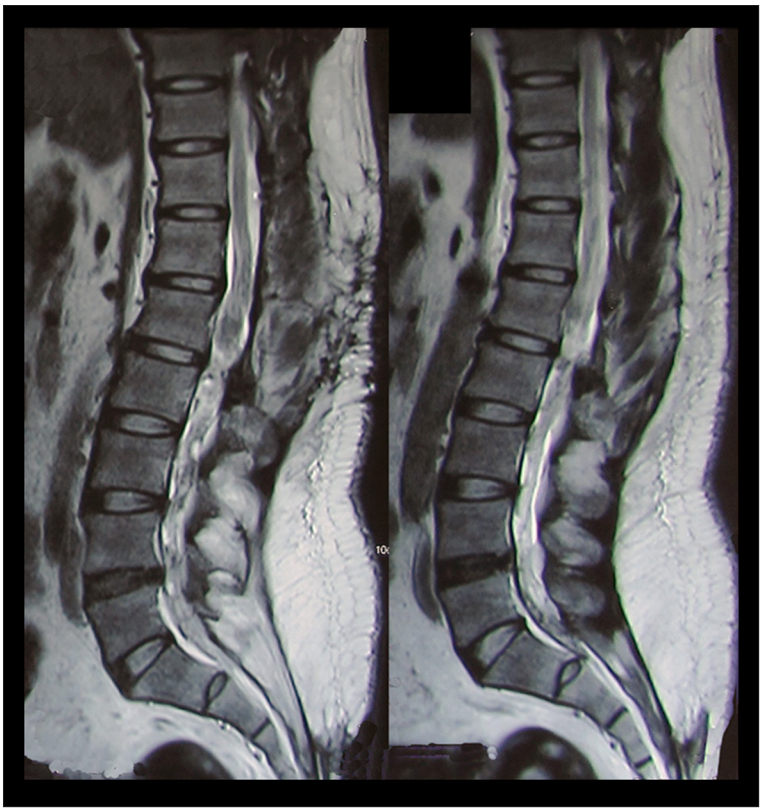

Figure 3. Magnetic resonance imaging at the final follow up (5 years after bone marrow derived mesenchymal stem cells injection) showing minimal changes of the myelomalacia and improvement of the transverse myelitis.

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significant changes between the initial examination and the final follow up.

Discussion

Chronic spinal cord injury (SCI) is currently associated with an irreversible morbidity due to lack of successful treatments despite various attempts over the past decade. Limited recovery of the nerve cells and unfavorable environment for healing impose major obstacles in SCI treatment. The primary mechanical injury directly disrupts the mechanical structure of the spinal cord such as axons, blood vessels, and cell membranes. However, it is during the sub-acute phase that the recovery inhibition occurs. The spinal cord nerve cells fail to adequately regenerate due to formation of glial scars, which act as mechanical and chemical blocks, as well as demyelination that is caused by oligodendrocytes apoptosis [5,7].

In chronic SCI, the glial scar tissues are already matured, characterized by cystic cavitation, myelomalacia, and occasionally syrinx formation. The aim of treatment in chronic stage is to encourage sprouting of disrupted axons, promote neuroplasticity, and remyelination of the previously demyelinated axons [7]. Current cell-based therapy is based on 2 main concepts: 1) directly replace the lost cells (axons and oligodendrocytes) and 2) influence the environment to support axonal regeneration [7].

As we used autologous bone marrow derived MSCs, problems associated with immunological reaction and ethical issue that frequently arise in allogeneic transplantation could be avoided [4,8]. Another advantages of bone marrow derived MSCs is that they are easier to harvest under local anesthesia, can be expanded well *ex-vivo*, and cause less morbidity associated with graft harvesting [8].

Determining which administration method to use is important in achieving a good outcome. Three main transplantation routes have been introduced: intravenous, intrathecal, and intramedullary injection [3,9]. Our method consisted of combining the direct parenchymal injection and multiple intravenous injections as the adjuvant. Direct parenchymal injection intramedullary is the best method in cell delivery in the animal model; however, when the study was translated to human cases, the risk of further injury to the spinal cord become a concern [10–12]. The main difference between subacute and chronic SCI is that, in the latter, the local inflammation process, which acts as the homing-effect-stimulator, is already resolved. This then results in failure of intravenous or intrathecal delivered stem cells in providing a significant neurological improvement in chronic SCI [13,14]. On the other hand, the result of direct parenchymal injection in chronic cases has shown promise. Park et al. [4] performed intramedullary MSCs injection at the center and the periphery of the lesion. Three out of 10 cases yielded in improvement of motor power in upper extremities [3]. Dai et al. [15] and Deda et al. [16] reported sensoric and motoric improvement in most of their chronic SCI patients treated by direct injection of BM-MSCs to the injured spinal cord. The differences between the aforementioned protocols and ours were principally in the surgical technique. In our protocol, the dural sac exposure to visualize the damaged cord was not performed to avoid the need of secondary dural repair or possible complication of iatrogenic cerebrospinal fluid leakage.

In order to further enhance the neurologic recovery, several adjuvant intravenous BM-MSCs injection were included in our protocol. Direct stem cell injection in the damaged cord will reinduce the homing signal from the site of injury. Homing signals such as cytokines, chemokines, and growth factors, will attract the systemically (intravenous) and locally (intrathecal) administered stem cell into the homing sites [17]. Engraftment efficiency after intrathecal delivery is significantly higher than intravenous delivery. Less prominent glial scarring and less host immune response are also found in the intrathecal delivery [9]. Adjuvant intravenous delivery was chosen ahead of intrathecal delivery since it was less invasive to be conducted in serial manner. Despite having a couple incidences of host immune response symptoms, most pf these symptoms were benign and resolved spontaneously.

Shroff et al. [5] reported the outcome of human embryonic stem cell administration in treating SCI cases. By using the homing signal hypothesis, intravenous and intrathecal administration resulted in improvement in neurologic function; however, there is still some debate regarding the presence of these homing signals in chronic SCI, not to mention diminished vascularity due to scar formation in these cases.

Based on our literature review of previous studies, currently there are a variety of approaches and techniques used in cell therapy for SCI. There were no standards in therapy approaches, as most of these approaches are still limited in small-moderate group clinical trials and although most of them reported positive trends in terms of motoric and sensory improvement, the amount of improvement was pretty diverse [18–20].

It is evident that the patient developed increased motoric function in 11 months after the first injection. However, we cannot determine how or why the improvement occurred in the more proximal lesion (L2 nerve root). The pattern and extent of initial injury, the length of previous denervation lesion, and remaining glial scar tissue might contribute to the re-innervation process. An important consideration for understanding and treating SCI in general is that each injury is unique.

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Further advances in diagnostic studies are also required to determine the extent and type of injuries, as it plays a significant role in understanding and treating chronic SCI injuries.

Conclusions

Based on its limited adverse reaction and neurological improvement, our case may serve as an additional alternative protocol in stem cell administration to treat chronic SCI patients. Further advances in diagnostic studies are also required to determine the extent and type of injuries, as it plays a significant role in understanding and treating chronic SCI injuries

Supplementary Files

Supplementary Video 1. Improvement of hip flexor muscle group showed by patients ability to crawl at the final follow up.

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