

Preliminary report on the safety, efficacy and functional recovery of spinal cord injury with autologous bone marrow derived mesenchymal stem cells – a clinical trial

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Abstract

Background: Cell based therapies are increasingly entering into clinical trials in the treatment of spinal cord lesions as it has been observed to help improve regeneration and spinal cord function. **Methods and Results:** Transplantation of autologous cultured bone marrow derived stromal cells (BMSCs) in 5 patients was attempted in the cases of total transectional spinal cord injury with respect to safety, efficacy, therapeutic time window, implantation strategy, method of administration, functional improvement. The report on the available data suggests that it is safe, efficacious and resulted in functional recovery in two patients. The follow-up examinations were done at 3, 6 and 12 months after cell infusion therapy by way of objective assessment in motor function and improvement based on ASIA score. No adverse reactions were encountered during the entire protocol. **Conclusions:** It is evident that the therapeutic time window, implantation strategy and post transplant care following injury will play an important role in Spinal cord Injury Management. BMMNC and BMSC dualistic therapy appears promising for faster healing of the scar and functional improvement. Trials involving a larger population are warranted before further conclusions can be drawn.

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INTRODUCTION

Human mesenchymal stem cells (hMSCs) derived from adult bone marrow represent a potentially useful source of cells for cell replacement therapy after nervous tissue damage. They are expanded in culture and reintroduced into patients as autografts/allografts. This is a preliminary report on early successful results obtained during treatment of five patients with chronic spinal cord injury (SCI). In the last decade, adult stem cells have preceded over investigational embryonic stem cell therapy due to their plasticity and possible differentiation into numerous cell types, including neural cells [1,2,3,4]. The two most important common findings are that the mesenchymal stem cells demonstrate significant promise in tissue regeneration and immune modulation. The limited therapeutic option in the management of severe neurological deficits with the resulting permanent invalidity requires new therapeutic approaches. Autologous bone marrow derived stem cells are

ideal candidates for treating SCI in emerging clinical studies, because there are no ethical obstacles to their use, and the health risk for patients with SCI is rather small. Numerous electrophysiological and histological preclinical studies have revealed that the implantation of stem cells from bone marrow or umbilical cord blood in animal models of SCI results in spared white matter and gray matter, neuronal and axonal regeneration, astrocyte proliferation, myelination, neovascularization, and functional improvement [1,6,7,8,9,10]. The reports on successful animal model experiments derive the scope of translation to human clinical trials. In this clinical trial, autologous cultured bone marrow derived mesenchymal stem cells were infused at the site of injury; the results of the 12-month clinical follow-up are described.

MATERIAL AND METHODS

Ethical approval for this study was obtained from the Institutional Review Board

(IRB) and Institutional Ethics Committee (IEC). Patients with traumatic SCI (Total Transectional Injury) with

complete motor and sensory disorder were enrolled for the study, and high-risk informed consent was obtained from each patient. The selection and exclusion criteria is summarised in Table.1

Figure 1

Table 1: Inclusion and exclusion criteria for the selection of patients.

| Inclusion criteria | Exclusion criteria |
|---|--------------------------|
| Paraplegic or quadriplegic patients with chronic spinal cord injury | Previous spine surgery |
| Patients 18-56yrs of age | Alcohol/drug abuse |
| No other associated primary disorder as pulmonary hypertension. | Psychiatric disturbances |
| No haematological/infectious diseases | No informed consent |

BONE MARROW ASPIRATES

Bone marrow aspiration was performed under aseptic precaution under General anaesthesia, from the Right iliac crest, and BM fluid was aspirated into a blood bag with prior addition of 6% Acid Citrate Dextrose (ACD). All patients tolerated the procedure very well.

MSC CULTURES

The bone marrow mononuclear cells (BMMNCs) were separated using Ficoll - hypaque (Sigma) gradient at 2500xg for 30'. The mononuclear cells were then plated at a density of 10 X10⁴ cells/cm² in Dulbecco's minimum essential medium (DMEM) supplemented with 10% Human Serum Albumin (Sigma, USA).

When cultures reached near confluence, cells were detached with 0.05% trypsin (Sigma, USA) and 0.53 mM ethylenediamine tetra acetic acid (Sigma, USA), and were passaged at a density of 1000 cells/cm². The adherent bone marrow stromal cells (BMSCs) were harvested at passage 2 for cell therapy. The culture medium was periodically checked for bacterial, fungal and mycoplasma.

STEM CELL THERAPY

Prior to the cell infusion, the confluent cultures were provided the niche with Brain Derived Neurotrophic Factor (BDNF) at 10ng/ml final concentration for 24 hrs. The cell concentrate was suspended in 2.0ml of filtered physiological saline and was infused under GA at the site of lesion. Patient was discharged from the hospital at 72 hours post-procedure after ascertaining that there are no adverse reactions. Follow up of the patient was done at 1 st month, 6th month and finally at 9 th month (total 3 visits).

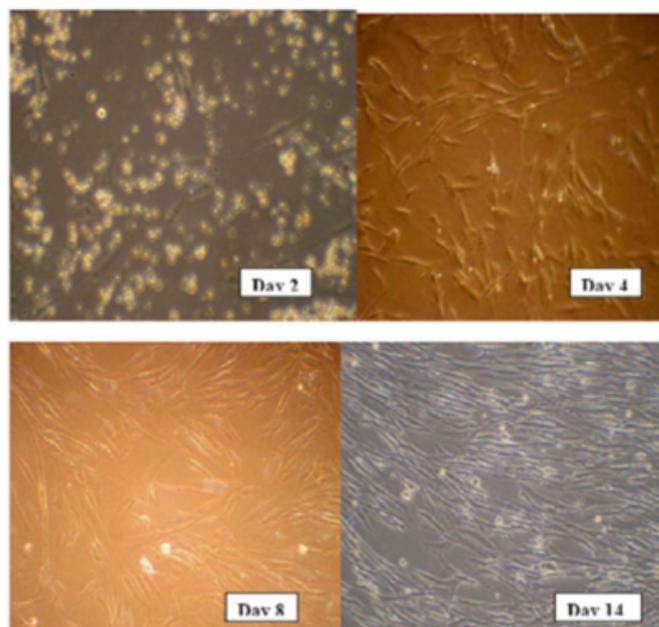
RESULTS

MSC CULTURES

Human bone marrow -derived MSCs were successfully culture -expanded from all donors. As reported by others, the cultures underwent an initial lag phase of about 5 days. A morphologically homogeneous population of fibroblast -like cells with more than 90% confluence was seen at a median of 13 days. After the first passage, the cells grew exponentially, requiring weekly passages (Fig.1). The cultures were contamination free.

Figure 2

Figure 1:MSC culture expansion on the day 2,4,8 and 14. Confluent cultures were obtained between 13 -15 day.

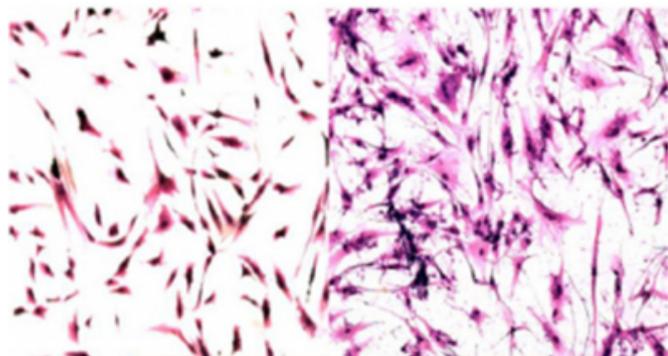


PHENOTYPIC CHARACTERIZATION

The cultured mesenchymal cells, isolated from second passage, were found to comprise a single phenotypic population for surface -expressed antigens by immunohistochemical analysis. MSCs were uniformly positive by IHC for Vimentin (Fig 2) and negative for CD34 (lipopolysaccharide receptor), CD 45 leukocyte common antigen), HLA -DR suggestive of non -contamination with haematopoietic lineage.

Figure 3

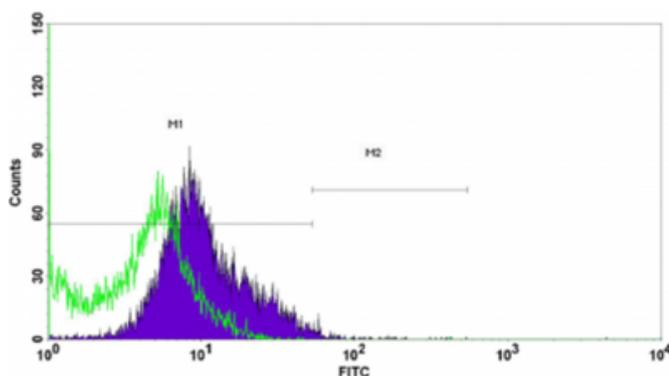
Figure 2: The stromal cells stained with H&E, Giemsa and Vimentin. They demonstrated positivity to Vimentin and negative to CD34&CD45;



The cells were immunophenotyped for the presence of CD106, a marker for mesenchymal stem cells by Flowcytometry. Briefly, the cells were incubated with 20µl of anti CD106 monoclonal antibody (BD Biosciences, USA) for 15 minutes at 4o C. The cells were washed off excess antibody and suspended in 250 µl of sheath fluid and the FACS caliber was run to demonstrate the positivity of CD106 marker along with IgG isotype control.

Figure 4

Figure 3: CD106 positivity of the bone marrow stromal cells. Green histogram represents isotype control and purple CD106.



Clinical details of the patients studied are summarised in Table 2.

Figure 5

Table 2

| Asia Grading | Interpretation |
|--------------|--|
| A | Complete: No motor or sensory function is preserved in the sacral segments S4-S5. |
| B | Incomplete: Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5. |
| C | Incomplete: Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3. |
| D | Incomplete: Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more. |
| E | Normal: motor and sensory functions are normal |

Volume of Bone marrow aspirated, mononuclear count, viability and CD106 positive count with the pre and post ASIA score are summarised in Table 3.

Figure 6

Table 3

| S.No | Age/Sex | Clinical History | Viability | Vol. of Bone marrow | MNC yield cells/ml | CD106 cells infused | Therapeutic Time window | ASIA Score | |
|------|---------|-----------------------------|-----------|---------------------|--------------------|---------------------|-------------------------|------------|----------|
| | | | | | | | | Before | 3m after |
| P1 | 27/M | L1 Burst with paraplegia | 98% | 60ml | 70x10 ⁶ | 10x10 ⁶ | 2yrs 8months | D | D ↑ |
| P2 | 26/M | D11 Burst with paraplegia | 97% | 60ml | 90x10 ⁶ | 6.5x10 ⁶ | 6yrs 7months | A | A |
| P3 | 43/F | Paraplegia | 98% | 60ml | 75x10 ⁶ | 6x10 ⁶ | 2yrs 7months | A | B |
| P4 | 38/M | D8-D9 burst with paraplegia | 96% | 60ml | 70x10 ⁶ | 9x10 ⁶ | 6yrs 10months | A | A |
| P5 | 38/M | D11 Burst with paraplegia | 99% | 60ml | 82x10 ⁶ | 11x10 ⁶ | 6yrs 7months | B | B |

After MSC cell infusion, there were no reports on the occurrence of pain, neuropathic irritation during the procedure and in the immediate post -procedure in any of the patients. Continuous patient monitoring was carried out during the first 24 hours post - procedure in an intensive care unit, with no alterations of these parameters.

The ASIA score and nerve conduction study reports are summarized in fig 4, fig 5.

Figure 7

Figure 4: The two representative cases with improved motor function have had mononuclear therapy elsewhere with a window period of 6 weeks prior to MSC therapy.

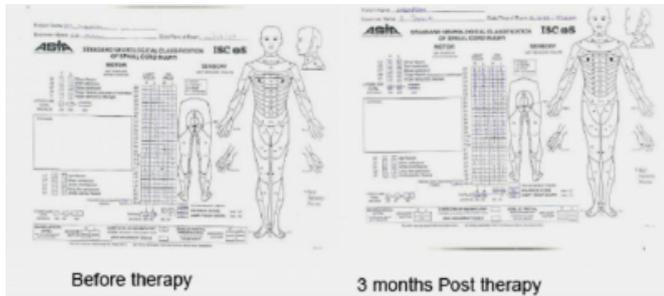
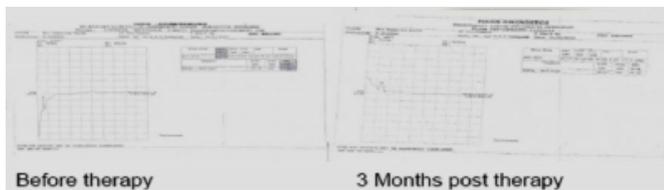


Figure 8

Figure 5: The nerve conduction study carried out before and 3 months post stem cell therapy.



DISCUSSION

The treatment approaches in SCI have not been successful with single strategy alone.

A number of studies report a poor prognosis; only about 5-6% of patients with complete SCI (ASIA grade A) improve after 1 year. Spinal cord injury represents a complex event, and therefore effective therapeutic strategies will consist of a series of interventions. First, secondary tissue loss should be prevented through early neuroprotective, anti-inflammatory, or immunomodulatory interventions. Subsequently, strategies to promote the regrowth of axons and the restoration of function will involve multiple approaches: reducing scar formation, overcoming additional inhibitory molecules, stimulating damaged nerve cells to regenerate axons, facilitating axonal growth across the site of injury, and enabling the formation of new connections.

Postnatal bone marrow has traditionally been seen as an organ composed of two main systems rooted in distinct lineages: the haematopoietic tissue proper and the associated supporting stroma – marrow stromal cells. Unlike haematopoietic stem cells, whose role in the treatment of haematopoietic diseases has been known for a long time. MSCs were originally examined only because of their critical role in the formation of the haematopoietic

microenvironment. Besides, the neuronal protective role of MSCs, haematopoietic stem cells also fosters neuroprotection. There is little doubt that bone marrow stem cells represent one of the most accessible sources of stem cells for therapeutic use. The ease with which they are harvested and the simplicity of the procedures required for their extensive growth in culture, together with easy expansion in vitro, may make them ideal candidates.

In our present study, the initial results are encouraging in that the two patients have demonstrated functional recovery in less than 2 months post transplantation. These two cases prior to admission at our hospital have had bone marrow derived mononuclear cell therapy elsewhere. The lag phase for MSC therapy included an average period of 6 weeks. The dualistic therapy of BMMNC and BMSC had significant benefit in the functional recovery as assessed by the masked scoring of ASIA and nerve conduction reports. The other group of patients persisted on the same ASIA score. The synergistic effect of mononuclear cells and mesenchymal stem cells provided with BDNF niche may prove to be a new approach to the treatment of SCI. The study presented by Moviglia et al., demonstrates the regeneration phenomenon based on the controlled inflammatory activity at the injured site [11]. Similar reports from Yaniv et al substantiates the contention that the local immune response plays a crucial role in recruitment of adult neural progenitor cells to the lesion site, and suggest that similar immunological manipulations might also serve as a therapeutic means for controlled migration of stem/progenitor cells to other acutely injured CNS sites [12]. The proposed study owing to no adverse events envisages a safe and effective transplant procedure. The method of administration at the site of lesion by various researchers documented varied success rates. Satake et al transplanted the MSCs into the subarachnoid space of the lumbar spine and reported the migration to the injured thoracic spinal cord tissue [13]. They have also reported the differentiation of MSC's to Nestin positive, immature neurons or glial cells in the rat model experimentation. The site-specific stem cell transplantation may be more advantageous for rapid myelination of axons with greater viability and number of cells available for the tissue repair and regeneration. The results of the present study concur with other reports for the therapeutic time window of 6 months to 12 months for the SCI therapy. MSC's transplantation within 12 months period has proved more effective as against greater time window with least possible success rates. Furthermore, there are several issues

that need to be addressed, such as implanted cell – host interaction in terms of immune response to transplanted cells, homing mechanism and differentiation to desired cell phenotype even before stem cell therapy being a routine clinical intervention. The dualistic therapy of olfactory ensheathing Glia (OEG) and MSC's are reported for accelerated myelination and functional recovery. The clinical study has been initiated at our centre is under evaluation.

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