Does Mesenchymal Stem Cell Therapy Help Multiple Sclerosis Patients? Report of a Pilot Study

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ABSTRACT

Background: Mesenchymal stem cells (MSCs) with their potential to differentiate into mesodermal and non-mesodermal lineages have several immunomodulatory characteristics. These properties make them promising tools in cell and gene therapy. **Objective:** To evaluate the potential therapeutic applications of autologous MSC in improving clinical manifestations of MS patients. **Methods:** Ten patients were included in this pilot study. All had progressive disease that had not responded to disease modifying agents including Mitoxantrone. Their Expanded Disability Status Scale (EDSS) score ranged from 3.5 to 6. Patients were injected intrathecally with culture expanded MSCs. They were followed with monthly neurological assessment and a MRI scan at the end of the first year. **Results:** During 13 to 26 months of follow up (mean: 19 months), the EDSS of one patient improved from 5 to 2.5 score. Four patients showed no change in EDSS. Five patients' EDSS increased from 0.5 to 2.5. In the functional system assessment, six patients showed some degree of improvement in their sensory, pyramidal, and cerebellar functions. One showed no difference in clinical assessment and three deteriorated. The result of MRI assessment after 12 months was as following: seven patients with no difference, two showed an extra plaque, and one patient showed decrease in the number of plaques. **Conclusion:** This preliminary report emphasizes on the feasibility of autologous MSC for treatment of MS patients. However, in order to draw a definitive conclusion a larger sample size is required.

Keywords: Mesenchymal Stem Cells, Multiple Sclerosis

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INTODUCTION

Multiple Sclerosis (MS) is an inflammatory autoimmune disease involving the central nervous system (CNS) and characterized by destruction of myelin sheaths and oligodendrocytes. This disease constitutes one of the most prevalent CNS disorders, with progressive and chronic destruction of the nervous system. Unfortunately, no definite treatment has been found for this disease. Even approved immunomodulatory therapies (IFN-β, glatiramer acetate and mitoxantrone) are only moderately effective in reducing disease exacerbations and brain inflammation (1). In animal studies, efforts to repair degenerated areas of the nervous tissue have shown improvements in the paralysis caused by MS (2).

In recent years, stem cell therapy has broadened the horizon of treatment for many disorders. Stem cells are multipotent cells which exhibit the ability to differentiate into many cell types. Adult stem cells can be found in many tissues such as bone marrow, liver, CNS, etc.

These stem cells can be isolated from adult tissues, expanded in vitro, and transfused into patients - a procedure used in stem cell therapy. It is postulated that the microenvironment of damaged tissues secretes factors that recruit stem cells to the site of active disease and enhances their differentiation into desired cells.

Among stem cells, Mesenchymal Stem Cells (MSCs) have attracted much attention due to their multi-lineage potential, immunosuppressive function, limited immunogenecity, and relative ease of growth and expansion in culture. Mesenchymal stem cells were initially believed to solely maintain the marrow stroma, necessary for hematopoietic stem cell (HSC) survival and function. Subsequent studies have convincingly demonstrated their differentiation into adipocytes, osteocytes, chondrocytes, tenocytes and skeletal myocytes under appropriate conditions, as well as neurons, astrocytes, and oligodendryocytes (3-6). In the presence of hMSC, immature or partially immature APCs are produced. Immature APCs shutdown T cells or induce an anergic state, either by eliminating T cells or modulating them toward a regulatory ($CD4^+CD25^+$) phenotype (7).

MSCs exhibit low immunogenecity due to the absence of cell surface HLA class II antigen(s) and co-stimulatory molecules such as CD80, CD86, and CD40 (8). Due to their immunosuppressive/immunomodulatory potential, MSCs down-regulate activated immune cell reactivity and thereby reduce tissue damage. MSCs play the role of stimulators and “cell factories” in injured and inflamed tissues when exposed to the local micro-environment. They promote tissue repair by differentiating into the injured cell types, thus compensating for their loss as well as secreting trophic factors (9). In this study, MSCs were separated from bone marrow of MS patients, expanded in culture medium (in vitro) and then injected intrathecally into patients. After injection, the safety of injection, clinical improvement of patients and, the degree of lesion repair in MS patients was assessed.

SUBJECTS AND METHODS

Ten patients (7 females, 3 males) with a mean age of 33 ± 5.90 years, and an EDSS of 3.5 to 6 participated in this study with their consent and approval of the ethical committee (FWA00001331). The patients had been diagnosed with MS for a period ranging from 3

to 21 years and a mean of 11.2 years. Eight patients were diagnosed with secondary progressive MS and two with primary progressive MS.

Inclusioncriteria Included. Expanded Disability Status Scale (EDSS) under 6, increased EDSS scores in the previous year, lack of serious familial disease, lack of response to other treatment options like corticosteroids, immunosuppressors (IVIg, Beta-interferons and Mitoxantrone), and an age under 60 years.

Sample Collection and MSC Expansion. Forty ml of BM was obtained from patients 2-3 months prior to injection. The BM mononuclear cells (MNCs) were separated by the Ficoll density gradient method. Vented flasks (75 cm²) with 21 ml MSC medium, consisting of Dulbecco's Modified Eagles Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (all from Gibco), were seeded with 1×10^6 MNC/ml for primary culture. Flasks were incubated at 37°C in a humidified atmosphere containing 5% CO2 and were fed by complete medium replacement every 4 days, until the fibroblast like cells at the base of the flask reached confluence.

On reaching confluence, the adherent cells were resuspended using 0.025% trypsin and reseeded at 1×10^4 cells/ml (1st passage). These were incubated again until confluence, and were once again trypsinized and re-seeded at 1×10^4 cells/ml. Passage was repeated until a required number of cells were achieved. The ability of isolated cells to differentiate to adipocytes and osteocytes was assessed as previously described (10).

By the end of final passage, when cells reached confluence, they were washed with tyrode salt and incubated with M199 medium for an hour. Cells were detached with trypsinization and washed with normal saline supplemented with 1% human serum albumin and heparin three times, then resuspended at a density of $1\text{-}1.5\times10^6$ cells/ml density.

Immunophenotyping. At the end of the last passage, surface expression of CD166, CD105, CD44, CD13 (MSC markers), CD34, CD45 (HSC markers) and CD31 (endothelial cell marker) were determined in culture-expanded MSCs. Anti CD44, CD45, and CD34 fluorescein isothiocyanate (FITC), anti-CD13 and CD31 phycoerythrin (PE) all from Dako (Denmark), anti CD166 FITC and anti CD105 PE were purchased from Serotec (Italy). Relevant isotope control antibodies were also used. Flow cytometry was performed on a FACScan (Becton Dickinson) and data were analysed with Cellquest software. Typical flow cytometry profile and Immunohistochemistry staining of MSCs isolated from this study are shown in figures 1-3.

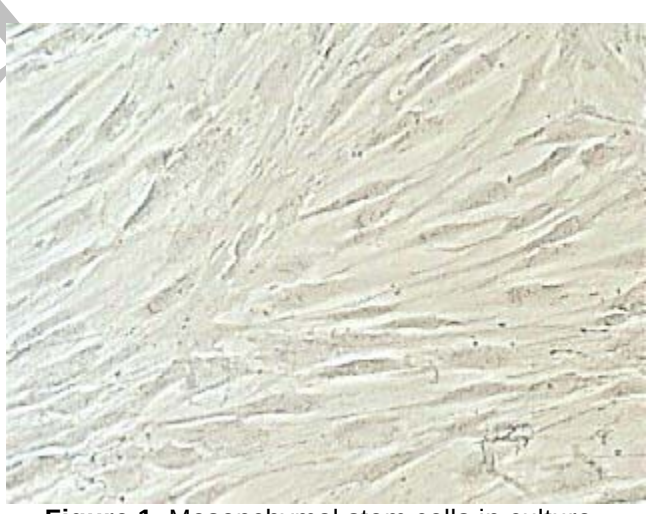

Figure 1. Mesenchymal stem cells in culture.

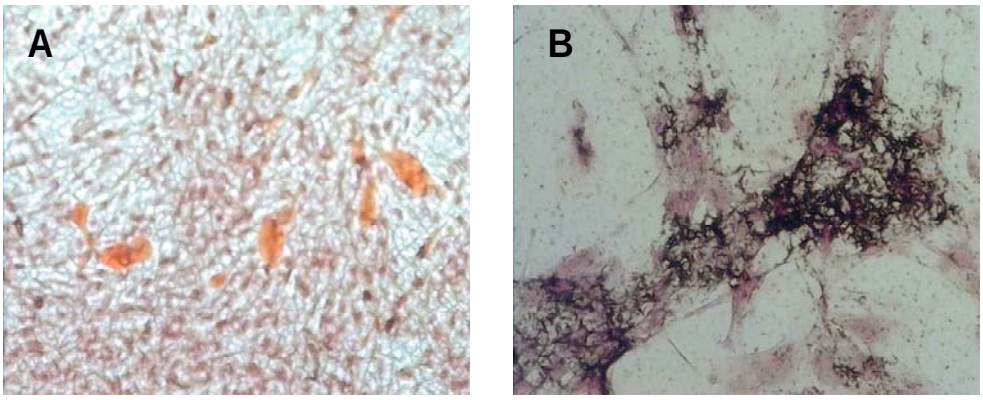

Figure 2. A) Oil red staining, MSC differentiation to adipocytes,
B) Van Kossas staining, MSC differentiation to osteocytes.

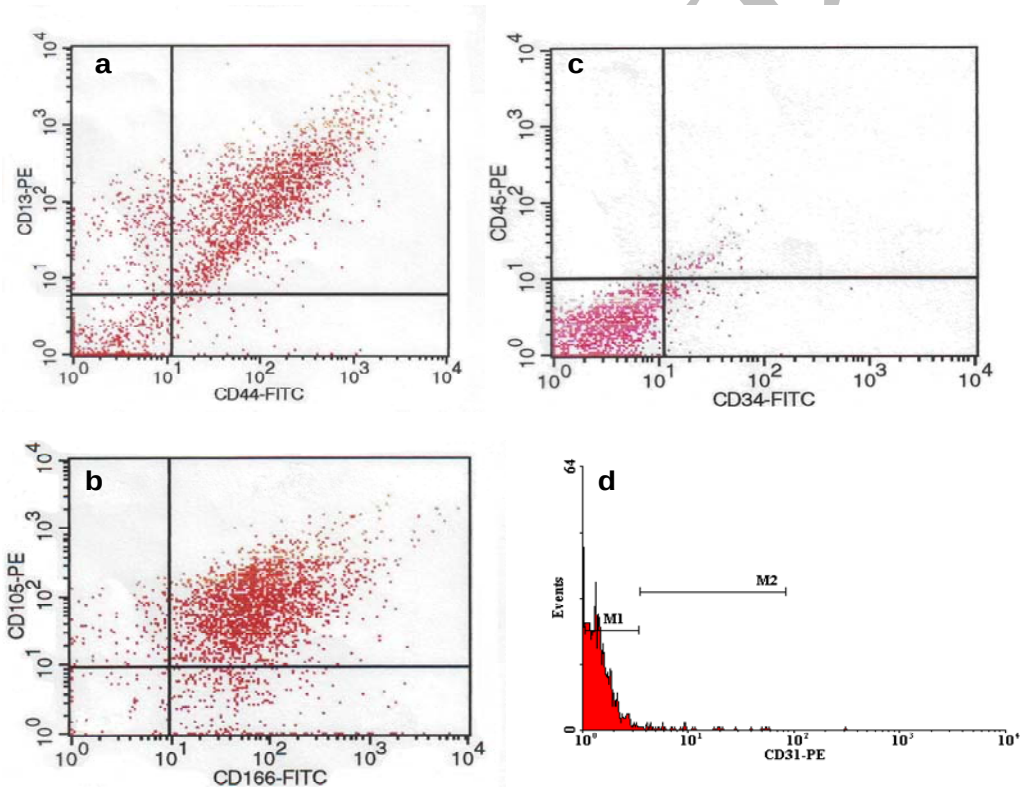

Figure 3. Flow cytometric analyses of MSCs: **a)** CD13/CD44 and **b)** CD105/CD166 positive but **c)** CD45/CD34 and **d)** CD31 negative.

Safety Assessment. To make sure that cells were not contaminated, bacteriological tests were performed on samples after each passage and at the time of injection. Viability of the cells was assessed by methylene blue dye exclusion test just before injection.

Injection of MSC. A mean volume of 5.5 ml containing 8.73×10^6 cells was prepared and injected intrathecally to each patient and observed for 4-5 hours before discharge. The patients were followed for a period of at least one year. The patients were not given any conditioning regimen or immunosuppressive drugs before or after MSC injection. The demographic characteristics and clinical data of patients are shown in Table 1.

Table 1. Demographic characteristics and clinical data of patients

Patients	Sex	Age	Diagnosis	Disease duration year	Injected cell No. $\times 10^6$	Month of follow up
1	F	30	SPMS	10	18	26
2	M	28	SPMS	7	8 + 13.2	26
3	M	28	SPMS	7	2.5	26
4	F	35	SPMS	16	2.26	26
5	F	39	SPMS	14	3.6	19
6	F	33	SPMS	21	13.2	18
7	M	39	PPMS	12	9.8	14
8	F	36	SPMS	9	7.2	14
9	F	40	SPMS	13	12.6	14
10	F	22	PPMS	3	5.75	13

EDSS and Functional System (FS) were evaluated monthly and MRI was performed prior to injection and 12 months after injection

RESULTS

At the time of injection, one patient was in an attack phase while others had attacks 2 months to one year (mean= 6 months) prior to injection. After injection, 9 patients experienced a slight headache, which was relieved by 3 doses of analgesics in two patients. One female patient was diagnosed with iatrogenic meningitis 4 hours after injection. A male patient experienced iatrogenic meningitis after his second injection (four months after the first injection). Both patients had no abnormalities in CSF and microbiological studies were also negative. Both patients received antibiotics for 14 days and discharged by the end of the second week without any serious problem. Six patients experienced new attacks 1 to 7 months (mean= 2.8 months) after injection (one patient had 2 attacks, while others experienced only one) which was treated with intravenous methylprednisolone.

EDSS improved in one patient by 2.5 points, no change was seen in four patients, while five had increased EDSS by 0.5 to 2.5 points (Table 2). Serial physical examination showed functional improvement by some degree in pyramidal, cerebellar and sensory pathways, and in bowel function of six patients. One patient had no change and four had worsened pyramidal, brain stem, sensory, bowel and cerebellar functions.

MRI assessment of 7 patients demonstrated no change in the size and number of plaques one year post MSC therapy. In one patient there was a decrease in the number of plaques in MRI, while another patient showed increased number of plaques. Enhanced lesions were observed upon MRI in another patient (Table 2).

Table 2. EDSS and MRI assessments of MS patients before and after MSC therapy

Patients	Months of follow up	EDSS score Before	EDSS score after Month 3	EDSS score after Month 12	Number of plaques in MRI before therapy	Number of plaques in MRI after therapy
1	26	6	6	7.5	SP*	NC***
2	26	5	4.5	2.5	SP	1 enhancing
3	26	5	5	5	SP	NC
4	26	5	5	5	FP**	Increase
5	19	6	6	6	SP	NC
6	18	6	7	8.5	SP	NC
7	14	5	5	6	SP	NC
8	14	4	4	5	SP	Decrease
9	14	6	6	6	SP	NC
10	13	3.5	3	4	SP	NC

DISCUSSION

Multiple sclerosis is a disease of unknown etiology with debilitating sequela and highest incidence between ages 20 and 40 years. In 1965, researchers discovered that myelin repair can happen without induction, probably due to activation of progenitor oligodendrocytes, causing spontaneous remyelination. They also found that PDGF plays an important role in multiplication and growth of these progenitor cells (11). Unfortunately, this spontaneous repair is very limited and therefore can not support reconstitution of damaged myelin and improvement in the patient's condition. Considering this, stem cells are a promising tool in the treatment of MS. Ben Hur et al injected mice with adult brain stem cells expanded in culture media, and observed improvements in the paralytic conditions of such mice (12). In 1998, ten cases of autoimmune diseases, including 2 MS patients, received autologous hematopoietic stem cell transplantation (HSCT) (13, 14). The prevailing concern in autologous HSCT is that lymphocytes originating from the injected stem cells can themselves be the cause of reemerging autoimmune condition in susceptible individuals. Under such conditions, allogenic HSCT is preferable to autologous HSCT. However, due to high mortality risk in allogenic transplantations, this alternative has not been clinically feasible up to now (15). Recent studies on allogenic transplants with modified conditioning regimens claim to be “highly encouraging”, but long term speculation remains to be answered (16).

Blanco et al reported the outcome of 250 autologous HSCT in MS patients (17),indicating that the procedure is effective in modifying the progressive course of the disease and deserves further assessment in the setting of randomized trials.

MSCs promote survival of neural cells by secreting neuron regulating proteins such as Brain derived neurotropic factor (BDNF) and Nerve growth factor-β (β-NGF) (18).

It has been reported that MSC differentiation is controlled by factors present in the tissue after transplantation (19, 20). On the other hand, recent studies demonstrated that MSCs act as immunomodulatory cells in different ways: 1-induce inhibitory phenotypes in APC following their activation, 2-Up-regulate the T_{reg} subsets and down-regulate differentiation of alloantigen induced lymphocytes, 3- decrease the incidence of acute GVHD and cure severe acute GVHD refractory to conventional immunosuppressive therapy, 4- down-regulate proliferation of CTL and inhibit NK cell activity (21-23).

Zappia and colleagues used autologous MSCs to treat EAE (experimental autoimmune encephalomyelitis), an animal model for MS. They showed that administration of MSCs successfully ameliorated severity of disease. They concluded that effectiveness of MSCs in the treatment of autoimmunity results from a profound suppression of effector T cells and induction of peripheral tolerance (24).

Several studies showed that activation of specific cells targeting myelin or myelin producing cells is involved in the pathogenesis of MS (25, 26).

Considering that MSCs have the potency to differentiate into neuronal cells and possibly repairing damaged tissue, and by taking into account the immunomodulatory effects of these cells, it is a very compelling hypothesis that transplantation of MSC cells into MS patients whether autologously or allogenically can be used as a tool for inducing reconstitution and improvement in disease condition by two means: First, tissue repair and second, decreased inflammatory responses. A recent study suggests that stem cells in general do not locally differentiate, but apparently remain in an undifferentiated state and exert a neuroprotective effect (27).

In our study, we observed that intrathecal injection of expanded MSCs is a feasible procedure with side effects similar to other regular intrathecal injections and therefore not related to MSC injection in particular. One of our patients had improved EDSS by 2.5 points within 12 months after MSC injection and four others showed some degree of improvement in their daily functions with no change in EDSS. Although five patients had a deteriorated EDSS by 0.5 to 2.5 points, it is worth noting that all of them felt better subjectively within 3 months after the beginning of the treatment.

In conclusion, intrathecal injection of MSC was not associated with any serious complications, rather it was associated with some improvement in some patients and halted disease progression in others. In five patients, MSC therapy had no effect on the course of the disease.

To clarify the role of MSC therapy in the management of MS, further investigations are necessary to determine the required cellular dose, number of injections, timing of injections, proper use of co-stimulators, determining the best cell subtypes (among stem cell population) and finding a non invasive way for labeling and tracking MSC cells.

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