

Mesenchymal Stem Cell Therapy for Multiple Sclerosis

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Abstract

Human mesenchymal stem cell (MSC) can be isolated from bone marrow (BM) and differentiated into multiple lineages. These properties make them promising tools in cell and gene therapy. Up to now, no definite therapeutic intervention for late stages of multiple sclerosis (MS) has been found. We decided to inject MS patients with autologous expanded MSC.

Five patients participated in this ongoing study. Patients were injected intrathecally with the culture expanded BM MSCs. Patients were followed monthly for their clinical status and every 3 months regarding their magnetic resonance imaging.

During 7 months follow up, one patient improved 1.5 EDSS, two patients improved by 1 and 2 scores, and two others remained unchanged till now. The first MRI findings of patients showed no change. We can claim that the injection of expanded MSC is a safe procedure. Three patients showed some degree of improvement and the other two had no progression. Patients should be followed for at least one year and a larger sample is required in order to draw a definite conclusion.

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Introduction

Multiple Sclerosis (MS) is an inflammatory autoimmune disease involving the central nervous system (CNS) which leads to the degeneration of the myelin sheath, the oligodendrocytes and the axons. This disease constitutes one of the most prevalent CNS disorders, progressive and chronic destruction of the nervous system. Unfortunately, no definite treatment has been found for this disease. Even approved immunotherapies have been of little use in limiting the acuteness and progression in some patients.⁽¹⁾ In animal studies, efforts to repair degenerated areas of the nervous tissue have shown improvements in the paralysis caused by MS.

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 (BM), liver, CNS etc. These days a visible notion of "self-repair" by a pool of progenitor cells has opened up an exciting new avenue of research orientation at the level of basic and preclinical studies. The

idea is that the stem cell pool is active and continually functioning toward the repair of inflamed or damaged body tissues, at sub-optimal levels. However, the number of these cells is so low that they can not effectively repair the damaged tissue. When these stem cells are isolated from adult tissues, they are expanded in vitro and transfused into the patients- a procedure used in stem cell therapy nowadays. At this time it is postulated that the microenvironment of the damaged tissue secretes factors that recruit stem cells to the site and enhance its differentiation into the desired cell types.

Among these stem cell, Mesenchymal stem cells (MSC) have attracted much attention due to their relative ease of growth and expansion in culture, and their ability to differentiate into mesodermal lineages as well as neurons, astrocytes, and oligodendrocytes.⁽²⁾ It has been shown that these stem cells have an immunosuppressive quality (nonimmunocompetency) as well.^(3,4)

In this study, we have separated MSC from the bone marrow of our MS patients and expanded these cells in culture medium (in vitro) and then

injected them back into the patients' intrathecally. After injection we assess the safeness of the injection, the patient's improvement from a clinical point of view and finally, degree of lesion repair in MS patients.

Materials & Methods

Until now five patients (3females, 2 males) with the mean age of 31 years participated in this ongoing study with their consent and approval of the ethical committee (FWA00001331). These patients have been living with MS an average of 8.2 years (between 6 to 15 years).

Including criteria was: Patient diagnosed with secondary progressive MS, expanded Disability Status Scale (EDSS) under 6, progressive EDSS in the past year, no serious familial disease, no response to other treatment options like corticosteroids, immunosuppressors (IVIg, Beta-interferons and Novantrone), and age between 15 and 60 years.

Sample collection and MSC expansion

25 ml of BM was obtained from the patients 2-3 months prior injection. The BM mononuclear cells (MNCs) were separated by the ficoll density gradient method. Vented flasks (75cm²) with 21 ml MSC medium, consisting of Dulbecco's Modified Eagles Medium (DMEM) with 10% Fetal Bovine Serum (FBS), and 10% 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 re-seeded at 1×10^4 cells/ml (1st passage). These were incubated again until confluence, and were once again trypsinizes and re-seeded 1×10^4 cells/ml. The number of passage of cells depending on the required amount of cells can be repeated. To make sure these are stem cells, we assessed their differentiation potential in the second passage culture. Adipogenic differentiation was assessed by incubating the cells with DMEM-LG and 10% FBS supplemented with 0.5 μ M hydrocortisone, 0.5 μ M isoboutyl methylxanthine, 60 μ M indomethacin, and 10 μ g/ml

insulin. Osteogenic differentiation was assessed by incubating the cells with DMEM-LG and 10% FBS supplemented with 0.1 μ M dexamethasone, 10 μ M β -glycero-phosphate, and 50 μ M ascorbate.

At the end of the last passage, when the 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 and resuspended at $1-1.5 \times 10^6$ /ml density. This washing process eliminates trace amounts of FBS as well.

Immunophenotyping

At the end of last passage, surface expression of CD166, CD105, CD44, CD13 - which are MSC surface markers - and CD34 and CD45 - which are HSC surface markers - were determined on culture-expanded MSCs. The monoclonal antibodies used were anti CD44, CD45, and CD34 fluorescein isothiocyanate (FITC), anti-CD13 phycoerythrin (PE) (all from Dako), and anti CD166 FITC and anti CD105 RPE (from Serotec). Relevant isotope control antibodies were also used. Flow cytometry was performed on a FACScan (Becton Dickinson) and data were analyzed with Cellquest software.

Safety Assessment

To make sure the cells are not contaminated, bacteriological tests were performed on the samples for every passage and at the time of injection. Viability of the cells were assessed by Methylene Blue dye exclusion test just before injection.

Injection of MSC

A mean volume of 5.5 ml containing 6×10^6 prepared cells were injected intrathecally to the patients. They were observed for 4-5 hours before being released from the hospital. Follow up of the patient condition will be one year.

The fallow up criteria is as follows:

- Evaluation of EDSS monthly.
- Assessment of MRI at 3, 9, and 12 months post injection.

Results

At the time of injection, none of the patients were in the attack phase. On the day cells were

injected, 4 of 5 patients had a slight headache, 3 got better without prescription but one used 3 doses of analgesic. The fifth one got iatrogenic meningitis 4 hours after injection, but she was treated for 14 days with antibiotics and discharged by the end of 14 days without serious problems.

This patient got pregnant 7 months after she received the injection. She underwent voluntary D&C in the 7th week of pregnancy although the sonography showed a healthy embryo. Two of the patients experienced attacks, one of them a month after the injection and the other 5 months after. Both of them were treated with pulse of steroids.

During 7 months follow up, the sensory function of one patient improved by 2 scores and the cerebellar function of another patient improved by 1 score. The third patient who was injected about 7 months ago had an EDSS 5. Now after 7 months his EDSS has decreased to 3.5 and clinical improvements in the sensory, cerebellar, and pyramidal scores have been noted as well. However, EDSS of the other patients remained unchanged.

MRI assessment of 4 patients showed no changes in the size and number of lesions in the first 3 months post MSC injection. Three patients did not manifest gadolinium enhanced lesions in their MRIs, but an enhancing lesion was observed beside the right lateral ventricle in one of the patients.

The fifth patient has not entered its 3rd month so no MRI has been taken yet.

The result of flow cytometry analysis for CD13, CD44, CD105, and CD166 was positive and for the hematopoietic markers, CD34 and CD45 were negative.

Results of bacteriological analysis were negative for all samples. The viability of injected cells were over 95 %. MSC differentiation to adipocyte and osteocytes was approved by Oil red O and Van Kossas staining, respectively.

Discussion

The onset of MS is generally in the range of 20 to 40 years and causes general disability in the people diagnosed with it. One of the greatest concerns is finding a treatment for this disease. Various methods have been proposed along with the better understanding of the mechanisms of disease. In 1965, researchers

discovered that myelin repair can happen without induction, probably due to the activation of present progenitor oligodendrocytes causing spontaneous remyelination. They also found out that PDGF plays an important role in the division and growth of the progenitor cells.⁽⁵⁾ Unfortunately, this spontaneous repair is very limited and can not support reconstitution and improvement in the patient's condition. Seeing the positive effect stem cells have displayed in the repair and reconstitution of tissues of different cells, studies for treatment of MS with their use attracted the attention of researchers. Ben Hur et al⁽⁶⁾ injected MS model mice with Adult brain stem cells expanded in culture, and observed an improvement in the paralysis condition of these mice. In 1998, ten cases of autoimmune disease, two of which were MS patients, received autologous hematopoietic stem cell transplantation (HSCT) and within 5-17 months 100% of the patients either improved or showed no signs of progression in the disease state. Their assessment was that HSCT in autoimmune patients is safe (7). The prevailing concern in autologous HSCT is that lymphocytes resulting from the injected stem cells can themselves be the cause of reemergence of the autoimmune condition in susceptible individuals. Under such conditions allogeneic HSCT is preferable to autologous HSCT. However, due to the high risk of mortality in allogeneic transplantations, this alternative is not applicable for the time being.⁽⁸⁾ In a review published recently, Blanco Y et al⁽⁹⁾ reported the outcome of 250 autologous HSCT and concluded that for MS patients this treatment is feasible in severe forms of MS provided strict eligibility criteria are applied to patients and centers. She believes that the procedure is effective in modifying the progressive course of the disease and deserves further assessment in the setting of randomized trials.

Other concerns pertaining to the issue of autologous transplantation include:

high risk of infection in the period of neutropenia, drug side effects, and the possibility of unaccounted for change or harm to the HSCT microenvironment imposed by the cytotoxic conditioning regimen which could lead to a decrease in the rate of nervous cell repair, as demonstrated by animal models.⁽¹⁰⁾ MSC are rare cells in the bone marrow stem cell popula-

tion. MSC found in bone marrow act as nurse cells in the growth, development, and differentiation of hematopoietic cells and can themselves differentiate into nonhematopoietic lineages like: osteoblasts, adipocytes, chondrocytes, myeloblasts, and neuronal cells⁽¹¹⁾ (neuron and purkinje cells.⁽¹²⁾ Studies have confirmed that injection of MSC into brain parenchyma results in migration, with high mobility, from the site of injection by known migratory routes of neuronal stem cells to inflamed and damaged sites.⁽¹³⁾

It has been reported that MSC differentiation is controlled by factors present in the tissue after transplantation.⁽¹⁴⁾ On the other hand, in 2002 Bartholemew et al showed that MSC cannot induce and repopulate allogenic lymphocytes in vitro and when they added MSC to the mixed lymphocyte reaction, up to 50% of lymphocyte division was inhibited. This group also demonstrated that if a baboon is injected with donor MSC prior to unmatched MHC skin transplantation, the survival of the skin graft increased markedly compared to controls.⁽¹⁵⁾ One study in France⁽¹⁶⁾ showed that MSC have immunosuppressive potential probably due to the induction of soluble factor secretion from CD8+ cells⁽¹⁶⁾.

Multiple studies showed that in the pathogenesis of MS, activated specific cells targeting the myelin or myelin producing cells are involved.^(17, 18) Knowing that MSCs have the potential to differentiate into neuronal cells and so have the possibility to repair damaged tissue, and adding to it the immunosuppressive potential of these cells, it is a very intriguing hypothesis that transplanting these cells into MS patients whether autologously or allogeneically could induce reconstitution and improvement of diseased condition by two means namely: tissue repair and decreased inflammatory response.

In our ongoing study, up to this point, can claim that the injection of expanded MSC intrathecally is a safe procedure and the side effects are similar to any regular intrathecal injection, therefore not related to MSC injection in particular. One patient improved 1.5 EDSS within 6 months; two others improved one by one score and the other by two scores. None of the patients displayed any change in their MRI, before and three months after injection. The good news is that all of our patients were cho-

sen from among non-responding progressive individuals, and even a stabilized or non-progressive state (which we have observed to this point) can be considered an improvement in their condition.

Finally, to find out about the role of these cells in myelin repair, patients should be followed for at least one year and a larger sample is required in order to draw a definite conclusion.

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