Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: A pilot study

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ABSTRACT

We explore the safety, and therapeutic benefit of intrathecal injection of ex-vivo expanded autologous bone marrow derived mesenchymal stem cells (BM-MSCs) in 10 patients with advanced multiple sclerosis (MS). Patients were assessed at 3, 6 and 12 months. Assessment at 3–6 months revealed Expanded Disability Scale Score (EDSS) improvement in 5/7, stabilization in 1/7, and worsening in 1/7 patients. MRI at 3 months revealed new or enlarging lesions in 5/7 and Gadolinium (Gd+) enhancing lesions in 3/7 patients. Vision and low contrast sensitivity testing at 3 months showed improvement in 5/6 and worsening in 1/6 patients. Early results show hints of clinical but not radiological efficacy and evidence of safety with no serious adverse events.

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1. Introduction

MS was first described by Charcot in 1868 (Murray, 2009). Even then, Charcot was frustrated by the resistance of this disease to all his treatments including electrical stimulation, strychnine and gold injections. Little did he know that 125 years will elapse before the first effective therapy becomes available. Although B-interferons were a major advance in the treatment of this disease, their effect is still limited (Vosoughi and Freedman, 2010; Grigoriadis, 2002) and they have not been convincingly shown to change the natural course of the disease. The more recent therapies including immunosuppressants, oral agents and monoclonal antibodies are promising but more data is needed concerning their long term safety profile (Caroll, 2010; Wiendl and Hohlfeld, 2009).

Two opposing factors determine the final residual damage in the central nervous system of MS patients: the damaging immunogenic processes and the intrinsic repair mechanisms (Krampera et al., 2006, Pluchino et al., 2009). An ideal treatment would reduce the abnormal immune response through immunomodulation, immunosuppression or other mechanisms, and enhance repair through boosting intrinsic repair mechanisms or even cell replacement (Martino et al., 2010). Most current therapies focus on the first mechanism although ample evidence shows that failure of repair mechanisms is a major contributing factor to the ultimate outcome in MS (Pluchino et al., 2009). In a recent post-mortem study of 51 MS cases, only 20% of the patients had evidence of adequate remyelination (60%–96% of the total lesion remyelinated) (Patrikios et al., 2006). Multiple factors contribute to this remyelination failure: oligodendrocyte precursor cells (OPC) attrition or failure to differentiate, astrogial scaring forming a physical barrier to OPC’s migration towards demyelinating areas, and inhibitory molecules that prevent migration of progenitor cells and remyelination (Payne et al., 2008).
Treatment with autologous stem cells (whether embryonic or adult in origin) might be effective through multiple mechanisms including neuroprotection, immunomodulation and neuroregeneration (Ucelli and Mancardi, 2010). The therapeutic use of embryonic stem cells in patients with MS is hindered by ethical issues and the potential risk of teratoma formation (Karussis and Kassis, 2008b). Adult stem cells are multipotent progenitor cells present in different tissues like bone marrow, adipose tissue, olfactory bulb, central nervous system and others (Pluchino et al., 2007). Animal experiments in the experimental allergic encephalomyelitis (EAE) mouse model of MS showed that intravenous, intraperitoneal or intravenous injection of human or murine BM-MSCs significantly improved clinical outcomes (Zappia et al., 2005, Bai et al., 2009, Gordon et al., 2008, Zhang et al., 2005, Kassis et al., 2008). A phase I trial was initiated to evaluate the safety and feasibility of intrathecal injection of autologous BM-MSCs in MS patients.

2. Methods

2.1. Clinical

The Institutional Review Board at the American University of Beirut Medical Center approved the study protocol and the informed consent. Subjects were enrolled if they fulfilled the following inclusion criteria: diagnosis of definite MS as per the revised McDonald criteria (Polman et al., 2005), age between 18 and 65 years, failure of medical therapy defined as progression to advanced disability inspite of one or more standard MS therapies, and major disability as defined by EDSS of 4.0 to 7.5. Patients were excluded if they had a clinical relapse within 30 days prior to enrollement or if they suffered from any bone marrow disease, glaucoma or any other potential condition that could affect retinal nerve fiber layer measurement. Enrolled patients signed an informed consent for participation in the trial and underwent bone marrow aspiration (20 ml) from the posterior iliac crest under local anesthesia. The ex-vivo expanded cells were injected in 5 ml of sterile saline solution under local anesthesia using a 20 gauge spinal needle into the subarachnoid space at C1 – C2 and L2 – L3 disc space levels under fluoroscopic guidance.

Patients underwent a clinical assessment at baseline, 3, 6 and 12 months post-injection including EDSS and Multiple Sclerosis Functional Composite (MSFC) scores.

2.2. Isolation and in-vitro expansion of BM-MSCs

Bone marrow mononuclear cells were isolated by Ficoll density centrifugation. Mononuclear cells (1 × 10^6/ml) were cultured in Dulbecco’s modified Eagles medium (1000 mg/l glucose, L-glutamine and sodium pyruvate) (Sigma Aldrich, USA), with 1% Penicillin/Streptomycin, 10% fetal bovine serum (Gibco BRL, USA) at 37 °C in 95% air with 5% carbon dioxide at 100% humidity. The medium was replaced every 2 days. BM-MSCs that developed into colonies within 10 to 14 days were harvested using 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) and expanded (for 3 to 5 weeks) for a total of 4 to 5 passages to reach 3 to 5 × 10^6 cells/patient. On the day of injection, BM-MSCs were harvested using trypsin, washed with normo-saline, resuspended in 10 ml normo-saline, and infused into the patient (5 ml intrathecally and 5 ml intracranially). Cell viability was determined using trypan blue staining at the end of the harvest and before infusion. Cell cultures were tested for sterility by bacterial and fungal cultures at the initiation of BM-MSCs culture and before infusion. Finally, because BM-MSCs are highly likely to be differentiated, the gated cells were tested using flow cytometry on passage 3, and the stem cells were identified before infusion through a panel of antibodies as being CD34 and CD14 negative while CD29,CD44 and CD166 were positive. The stem cell nature was also tested functionally by demonstrating in-vitro differentiation capacity into osteoblasts and chondrocytes.

2.3. Laboratory tests

A 1.5 Tesla (T) MRI (Philips, Interas Holland) was used for imaging. The following tests were performed at baseline, 3 and 12 months post-injection. The brain imaging included: Dual Transverse T2 spin-echo weighted images (WI) (repetition time msec/echo time msec, 2900/120 for T2WI and 2900/20 for proton density WI), axial T2 fast fill echo (FFE) (483/23), axial T1–WI (598/15) pre- and post-contrast administration. The slice thickness was 3 mm and the field of view (FOV) was 25 cm. A single voxel magnetic resonance spectroscopy (MRS) was performed using TE = 136 msec and TR = 2000 msec. The voxel size was approximately 25 × 25 mm. 2 voxels were selected, one placed in the centrum semi-ovale and one in the midline including the corpus callosum and surrounding white matter. The peak area ratio of N-acetyl aspartate (NAA) over creatine (Cr) (NAA/Cr) was calculated. Optic nerve coronal T2 FFE (483/23) and coronal spectral inversion recovery (SPIR) gadolinium (Gd) (400/11) were performed, slice thickness = 3 mm and FOV = 2.05 cm. Cervical spine images included: sagittal short tann inversion recovery (STIR) (2500/60, IR = 170), sagittal T1–WI (400/10), Axial T2–FFE (400/16/11), sagittal T1–WI post-Gd (400/10) and axial 3D T1–WI post-Gd (9.3/4.6).

2.4. Ophthalmic assessment

Optical coherence tomography (OCT) and Sloan contrast sensitivity visual testing were performed at baseline, 3 and 12 months post-injection. After an initial complete ophthalmic evaluation, patients were screened for other possible causes of vision loss and retinal nerve fiber layer (RNFL) changes. Best corrected visual acuity was recorded using the distance early treatment diabetic retinopathy study (ETDRS) charts at 4 m. Low contrast sensitivity was assessed using the Sloan contrast sensitivity charts at 2.5% and 10%. After pupillary dilatation with 1% mydriacyl and 10% phenylephrine the nerve fiber layer (NFL) thickness was measured using the Zeiss Stratus OCT, version 4.0.1 software performing the fast RNFL tests 3 times then taking the average.

3. Results

Ten patients fulfilled the inclusion criteria and were enrolled in the trial (Table 1). They all showed evidence of disease progression during the year preceding enrollment. Three patients (7, 8, 10) failed to grow an adequate number of BM-MSCs (<2 × 10^6), a 30% failure rate. In all 3 patients, bone marrow aspiration was performed twice with the same result, suggesting an inherent bone marrow problem. There was no difference between those patients and the rest of the group with respect to age, disease course, previous interferon intake, immuno-suppressant therapy, bone marrow disease or disease duration.
The only major adverse event was a transient encephalopathy with seizures in patient 1, occurring few days after cell injection. He required hospitalization and intravenous valproate, but recovered without significant sequelae. Patient 3 had transient cervical and low back pain for few days without fever or meningeal signs.

None of the 7 patients treated was on concurrent MS therapies or received steroids during the trial period. Most patients reported subjective and functional improvement in their neurological status 3–6 months after the procedure (Fig. 1, Table 2). Follow-up assessment at 3–6 months is currently available on all 7 patients treated. The EDSS improved by 0.5–1.0 in 5/7 patients, was unchanged in 1 patient, and worsened by 0.5 in 1 patient. One year data available on 6 patients showed EDSS improvement in 3 by 0.5 and stabilization in 3 compared to baseline. It is of note that one of the patients that worsened after initial improvement at 6 months (patient 5) did so due to multiple osteoporotic vertebral fractures requiring two surgical interventions, thus affecting significant sequelae. Patient 3 had transient cervical and low back pain for few days without fever or meningeal signs.

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Vision and contrast sensitivity testing, available at 3 months on 6 patients, showed improvement by 1–3 lines in 5/6 and worsening by 1 line in 1/6 patients (Table 3). RNFL thickness measured by OCT, available on 4 patients, was unchanged in 3/4 and decreased by 24% in 1/4 patient. In 4 patients with 12 months follow-up, vision and contrast testing showed further improvement in 2 stabilization in 1 and slight worsening in 1 who was still improved compared to baseline. The RNFL thickness was unchanged in 3 patients with 12 months of follow-up OCT.

MRI data at 3 months revealed new or enlarging lesions in 5/7 and Gd + lesions in 3/7 patients. MRS was available on 6 patients and revealed a decrease in the NAA/Cr ratio by a mean of 0.18. In 4 patients with 12 months follow-up, vision and contrast testing showed further improvement in 2 stabilization in 1 and slight worsening in 1 who was still improved compared to baseline. The RNFL thickness was unchanged in 3 patients with 12 months of follow-up OCT.

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Figure 1. EDSS change in the 7 treated patients over 12 months. *EDSS assessed between 3 and 6 months. † Patient had multiple vertebral fractures secondary to osteoporosis which affected her ambulation.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Course</th>
<th>Age(years)</th>
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</table>

* Previous or current. F = female, M = male, SPMS = secondary progressive MS, RRMS = relapsing remitting MS, BINF = beta-interferon, MTX = mitoxantrone, AZA = azathioprine, CPX = copaxone, MET = methotrexate.

Table 2

<table>
<thead>
<tr>
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<th>6 months</th>
<th>12 months</th>
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Table 3

<table>
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</thead>
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<td>1</td>
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<tr>
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<td>Baseline 36 – +2/2</td>
</tr>
<tr>
<td>3</td>
<td>Baseline 33 -/20/60</td>
</tr>
<tr>
<td>4</td>
<td>Baseline 34 – +5/2</td>
</tr>
<tr>
<td>5</td>
<td>Baseline 35 – +1/1</td>
</tr>
<tr>
<td>6</td>
<td>Baseline 36 – +1/0</td>
</tr>
<tr>
<td>7</td>
<td>Baseline 37 – +1/2</td>
</tr>
</tbody>
</table>

* Number of lines improved compared to baseline.

** Visual acuity tested due to baseline finger counting vision.
the 4 patients with data at 1 year, one patient only showed a single Gd+ lesion. Three patients had MRS available at 12 months: 2 showed stabilization and the other further decrease in the NAA/Cr ratio.

4. Discussion

To the best of our knowledge this is the first published phase I clinical trial using autologous ex-vivo expanded BM-MSCs to treat patients with advanced MS. Thirty percent of patients failed to grow an adequate number of BM-MSCs (<\(2 \times 10^6\)) despite repeated bone marrow aspirations reflecting an inherent deficiency of such cells in the bone marrow of those patients. This inability to generate stem cells was not associated with age, sex, disease duration or previous/current treatments. The only other study to harvest and grow BM-MSCs in MS patients was by the Karussis group but they did not report on the number of cells obtained from different patients (Slavin et al., 2008). Studies in healthy subjects showed great variability in the number of BM-MSCs obtained from different donors and form the same donors at different points in time (Phinney et al., 1999). In the same regard, Mazzini et al. reported a 20% failure rate in BM-MSCs proliferation in patients with amyotrophic lateral sclerosis (ALS) (Mazzini et al., 2010). Due to the small number of patients in our trial however, a negative effect of previous therapies such as interferons or immunosuppressants cannot be ruled out. The exact reason behind such deficiency in certain MS patients is unclear and will need further studies to elucidate.

The only major side-effect was transient encephalopathy and seizures in patient 1 who was injected with the highest number of BM-MSCs (100 \(\times 10^6\)). The remaining 5 patients received 32–52 \(\times 10^6\) cells without major adverse events although the same culture techniques, culture medium and injection procedure were used in all patients. It is well known that cells are unstable in the cerebrospinal fluid leading to cell lysis and release of breakdown products which might elicit an inflammatory reaction. We postulate that the transient encephalopathy and seizures in patient 1 were secondary to lysis of high numbers of injected cells within the cerebrospinal fluid, especially that 50% of the cells were given through a cisternal puncture. Karussis reported a similar adverse event in 1/11 patients injected intravenously and intrathecally with autologous BM-MSCs (Karussis et al., 2008). They attributed the problem to inadequate washing of the Dimethylsulfoxide (DMSO) reagent used in BM-MSCs cultures at the hands of a new technician. Although this is a pilot study with small numbers aimed mainly at determining feasibility and safety of the procedure, few observations can be made on potential efficacy. All patients had evidence of progression in the year preceding enrollment in the trial, yet at 3–6 months post-treatment 6/7 patients showed improvement on different components of the EDSS and MSFC. Clinical improvement was maintained overall at 1 year for the 4 patients with longer follow-up except for the one patient with vertebral fractures.

Despite the clinical improvement or stabilization, MRI showed evidence of disease progression by an increase in lesion number or size (5/7), new enhancing lesions (3/7) and a decrease in NAA/Cr ratio in most patients. This increase in lesion number, size or enhancement could be due to failure of BM-MSC treatment in preventing ongoing inflammation related to disease activity.

On the other hand, it could be related to the immunologic effect of stem cells or repair mechanisms within the central nervous system or due to the reactive inflammation secondary to cell lysis byproducts. Visual assessment revealed an early improvement in contrast visual testing by 3 months in 5/6 patients which was partially lost by 12 months in 1/4 patients followed for that period. The RNFL thickness on OCT which reflects axonal loss within the optic nerve remained stable for the length of the study in 3/4 patients. Only patient 4 showed worsening of his vision and RNFL by 3 months, which correlated in his case with the highest number of enhancing lesions on MRI, indicative of persistent inflammatory disease activity.

Karussis and his group used BM-MSCs to treat ALS and MS patients (Karussis et al. 2008a, Karussis and Kassis, 2008b). They used a combination of intravenous and lumbar intrathecal injections and demonstrated a safety profile similar to ours, with only one major adverse event: transient asptic meningitis. Although objective measures such as MRI and OCT were not used, they reported clinical improvement in most of their patients. Another phase I/II trial using unmanipulated bone marrow stem cells injected intravenously is underway in Bristol (Scolding et al., 2008). Mazzini et al. transplanted BM-MSCs surgically into the thoracic spinal cord of 10 ALS patients in a phase I trial (Mazzini et al., 2006). They reported no major adverse events but the downhill course of the disease was unchanged. A phase I trial of autologous BM-MSCs stereotactically transplanted into the striatum of Parkinson patients is currently recruiting. Cells are expected to grow up into dopamine secreting neural cells (Shihabuddin and Aubert, 2010). Furthermore, the Food and Drug Administration (FDA) has recently approved a therapeutic pilot trial of intrathecal injected fetal neuronal cells in patients with ALS. The major concern in such early phase I trials is always safety (Talan, 2009). Our results show a good safety profile up to one year. However, our numbers are small and long term side-effects occurring beyond one year were not assessed. Intravenous injection of bone marrow stem cells has been used for years on a large number of patients with different hematological malignancies and has shown a good safety profile (Scolding et al., 2008), although those cells originated from minimally manipulated bone marrow and were not cultured under in-vitro conditions as in our trial. More recently intrarterial injection of bone marrow derived progenitor and mononuclear cells has been used to repair cardiac damage in acute myocardial infarction without significant major adverse events (Assmus et al., 2006; Lunde et al., 2006; Schachinger et al., 2006). There is also accumulating data on the safety of mesenchymal stem cell injections in various conditions such as diabetes mellitus type I, kidney disease, osteogenesis imperfecta and inherited leukodystrophies (Couri et al., 2006; Watorek and Klinger, 2006; Horwitz et al., 1999; Horwitz et al., 2001, Koc et al., 2002). Many more questions need to be answered before BM-MSCs are considered for clinical use: what is the best route of administration? Is it intravenous, intrathecal lumbar or cisternal? The only study comparing intravenous and intraventricular routes of administration of BM-MSCs in EAE showed that direct injection into the ventricles induced a more pronounced reduction in infiltrating cells and increased preservation of axons (Kassis et al., 2008). What is the number of injected cells needed to achieve our therapeutic goals? Should we use it in patients with advanced disability only, or in relapsing remitting cases at an earlier stage, when therapeutic interventions are usually more effective? What is the exact mechanism of action of BM-MSCs in MS? Is it a combination of immunomodulation, neuroregeneration and neuroprotection and is there any solid evidence for actual cell replacement? Does immunomodulation act only through peripheral lymphoid organs as shown by Gerdoni and Zappia, or is there a local immunologic effect within the central nervous system (Gerdoni et al., 2007; Mazzini et al., 2010)? Is neuroprotection better achieved by intrathecal injection and direct delivery of cells into the central nervous system especially in the cisternal area? How frequently should this therapy be given to maintain its effect in a chronic disease such as MS? Our trial’s main limitation is the small number of patients and its open-label unblinded and uncontrolled design which hinder any definitive conclusions about efficacy. All questions raised above cannot be answered except through an international concerted effort, leading to a phase II controlled trial with sham infusions, and objective measures of improvement such as MRI, OCT, evoked potentials and others. A recent consensus meeting addressed the feasibility of this approach and will hopefully pave the way for such a trial in the future (Freedman et al., 2010).
In conclusion, we have shown that autologous BM-MSC transplantation into the cerebrospinal fluid of MS patients through lumbar and cisternal punctures is a safe procedure. A high number of cells might cause a transient encephalopathy probably secondary to reactive inflammation to cell lysis byproducts. Early signs of clinical improvement are seen in most patients by 3 months and maintained up to 1 year.

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References


