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Antonio Uccelli^{1,2,3}, Alice Laroni¹ and Mark S Freedman⁴

Abstract

The unmet need for therapies capable of repairing the central nervous system (CNS) damage occurring in many diseases including multiple sclerosis (MS) has sparked the interest of the neurological community for stem cell-based therapies. An exhaustive amount of preclinical data has shown that the intravenous administration of mesenchymal stem cells (MSC), a subset of progenitor cells isolated from many mesodermal tissues, effectively ameliorates experimental autoimmune encephalomyelitis (EAE), a model of MS, through the release of anti-inflammatory and neuroprotective molecules. Based on these results, several small pilot clinical trials in subjects with advanced MS have demonstrated that MSC administration is safe and provided an early signal of clinical effectiveness. The current aim of clinicians and scientists interested in the development of MSC-based strategies for the treatment of MS is to have the ultimate demonstration in large clinical trials that MSC can inhibit CNS inflammation and foster tissue repair as realized clinically, with functional recovery, or visualized by magnetic resonance imaging (MRI).

Keywords

Mesenchymal stem cells, experimental autoimmune encephalomyelitis, multiple sclerosis, neuroprotection, immunomodulation

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Introduction

Far from being curative, therapies approved for the treatment of multiple sclerosis (MS) are unsatisfactory for a significant number of patients. In subjects with relapsing–remitting (RR) MS, they are associated with tolerability issues,¹ incomplete control of inflammatory activity² and adverse events.^{3–5} Moreover, no therapies exist that can trigger repair of the irreversible neuronal damage, which is a hallmark of the disease and leads to a progressive accumulation of disability in most affected subjects, observed clinically in the secondary progressive (SP) phase of the disease. Finally, no treatment has yet been approved for the primary progressive (PP) variant of the disease. These reasons justify the search by the scientific community for an innovative treatment of MS that is safe and well tolerated, potentially able to control the inflammatory activity leading to neurodegeneration and, more important, to promote repair of the damaged central nervous system (CNS).

Adult stem cells have recently entered the scenario of MS therapies based on a robust amount of preclinical data supporting the possibility that they could fit well this profile.^{6,7} In particular, mesenchymal stem cells (MSC) are a promising treatment for MS based on their therapeutic plasticity, relative ease of isolation from different tissues and

expansion in vitro, and safety of in vivo administration, usually following intravenous (i.v.) injection.⁸ MSC are pluripotent precursor cells and are abundant in the bone marrow (BM) where they closely interact with hematopoietic stem cells, supporting hematopoiesis.⁹ They have been used in clinical settings since the 1990s, when the first studies in humans were performed under the hypothesis that the administration of MSC (either autologous or allogeneic) could promote the engraftment of transplanted hematopoietic stem cells (HSC) in subjects with malignancies.¹⁰ The possibility that MSC treatment could improve the outcome of genetic diseases with mesenchymal involvement was

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also supported by pioneer studies in children with *Osteogenesis imperfecta*.¹¹ Parallel to these early clinical trials, preclinical studies explored the possible use of MSC in other disease settings, mostly with the aim of testing the capability of MSC to restore damaged tissues through their ability to engraft in the CNS and their transdifferentiation, which was observed, to some extent, in models of myocardial infarction and stroke.^{12,13} However, early reports on the capability of MSC to inhibit T-cell proliferation and to modulate immune responses^{14–16} provided a novel rationale to use MSC for the treatment of immune-mediated diseases, such as steroid-resistant graft-versus-host-disease (GvHD), in which a phase two clinical trial demonstrated that MSC administration was safe and effective.¹⁷ Based on the hypothesis that MSC could inhibit the pathogenic autoimmune response against myelin antigens, i.v. injection of MSC in mice with experimental autoimmune encephalitis (EAE), an animal model of MS, led to a significant amelioration of disease, inhibition of demyelination, and preservation of axons.^{7,18–20} These effects resulted from the induction of immune tolerance and the release of molecules fostering tissue repair, with little evidence of engraftment in the CNS. The importance of soluble mediators released by MSC in mediating the beneficial effect observed in EAE was strikingly highlighted by a recent study showing that the i.v. injection of conditioned medium from MSC cultures suffices to improve EAE scores and induce remyelination and tissue repair.²¹ However, it must be emphasized that MSC require being primed by inflammatory cues to acquire their maximal immunosuppressive phenotype.²² Thus, we could speculate that MSC, upon encountering inflammatory signals, behave like endocrine cells, mediating their therapeutic effect through release of immunomodulatory and neuroprotective molecules in the bloodstream.²³

This is the encouraging scenario arising from preclinical studies, which supports MSC clinical translation into an attempt to treat MS. In the following paragraph, we will review the clinical studies performed so far in MS and the perspectives in this field.

Mesenchymal stem cells for MS treatment: Clinical studies

To date, four clinical reports on the treatment of MS with MSC have been published.^{24–27} All studies had an open-label design and employed autologous MSC. The studies differed in way of administration, dose of MSC, and characteristics of the cohort, as detailed below. Outcomes were exclusively safety in all but one study, where efficacy was measured on changes in visual function.²⁴

Source of MSC

The utilization of autologous MSC provides several theoretical advantages. Firstly, it minimizes the risk of

transmission of infectious diseases. Secondly, although even xenogeneic MSC have proven effective in preclinical models¹⁹ and allogeneic MSC have already been successfully used for the treatment of diseases such as GvHD,¹⁷ some studies have shown the possibility that they might be rejected by the host immune system, therefore being less effective.²⁸ Importantly, MSC from subjects with MS display a normal phenotype and are fully functional in terms of proliferation, *in vitro* differentiation and immunosuppressive ability.²⁹

Route of administration

In pioneer clinical studies published so far, MSC were administered only intrathecally,^{25–27} intrathecally plus intravenously,²⁵ or exclusively intravenously.²⁴ The rationale for intrathecal administration was to deliver the cells directly into the CNS, thereby overcoming the limited amount of cells engrafting upon i.v. administration and increasing the total yield at the site of damage. In theory, local delivery may enhance MSC ability to promote repair by secreting neurotrophic factors, such as brain-derived neurotrophic factor (BDNF)³⁰ and antioxidant molecules.³¹ Some groups have also shown that MSC could trans-differentiate into neurons³² or promote neurogenesis through recruitment of local precursor cells.³³ However, it should be emphasized that most beneficial results observed in EAE were achieved following i.v. administration, which was sufficient not only to induce peripheral tolerance to myelin antigens,⁷ but also to preserve axons^{18,20,34} and foster remyelination.^{33,35} Moreover, the recent demonstration that soluble molecules released by MSC recapitulate all the beneficial effects observed when MSC are injected in mice with EAE further minimizes the importance of CNS engraftment.²¹ More important, a recent study comparing i.v. versus intrathecal administration in mice with EAE demonstrated similar effects on clinical and histological parameters.³⁶ In addition, intrathecal administration in humans is invasive and may lead to meningeal irritation.^{25,26} In one case, transient acute encephalopathy with seizures, likely related to CNS irritation, was reported in a subject who had received a high dose of MSC intrathecally.²⁷ Based on these considerations, i.v. administration of MSC should be considered preferable over intrathecal delivery.

Dose of MSC

Most preclinical studies in murine models employed a single dose of 1×10^6 MSC. Translated into a clinical setting, this would amount to a dose of more than 50×10^6 MSC/Kg, considering that when treated for EAE, mice are aged six to eight weeks and weigh about 20 grams. Unfortunately, it is almost impossible to recover and *in vitro* expand such quantity of MSC from a standard BM aspiration. Therefore, doses of MSC used in clinical studies with MS patients

have been considerably lower. There is considerable variability among studies in the numbers of injected cells, which depended on cell recovery from BM harvest and were scaled according to the patient's weight.^{24–27} The same variability was reported in other clinical settings; however, a mean dose of about $1–2 \times 10^6$ per Kg of body weight was chosen in pivotal trials with hematologic diseases¹⁷ and in patients with other immune-mediated diseases such as Crohn's disease.³⁷

Indication for the different phases of MS

Studies have focused, so far, on subjects with progressive MS,^{24,26,27} or with active disease not responding to approved treatments.²⁵ As we previously mentioned, available treatments have a poor impact on disease course during the progressive phase, when inflammation fades and neurodegeneration leads to disability. A possible explanation is that other mechanisms, in part independent from early inflammation mainly mediated by cells of the acquired immunity, may lead to neurodegeneration; this could arise from an exhaustion of the properties of self-repair by local precursor cells harmed by chronic inflammation, and consequent degeneration of axons and neural cells. The optimal goal for the utilization of adult stem cells such as MSC would be to impact on the progressive disease course by modulating chronic compartmentalized inflammation, preserving neurons from degeneration, and promoting tissue repair. Indeed, the recent trial published by Connick et al. demonstrated the effectiveness of i.v.-administered MSC on visual parameters in progressive patients (as further detailed below).²⁴ However, the ability of MSC to translate their reparative features observed in EAE into a clinically relevant impact on the progressive phase of the human disease is yet to be demonstrated and will require properly designed clinical studies. The scenario of early relapsing–remitting MS is fortunately different because several drugs are approved for such disease phase and can significantly impact on relapse rate by modulating the function of auto-aggressive peripheral immune cells before they cross the blood-brain barrier and mediate disease progression. However, there are still a number of patients with aggressive disease who do not respond to such therapies or are at risk for serious adverse events. Due to the well-established ability of MSC to inhibit inflammation and pathogenic autoreactive cells, it is conceivable to propose MSC as a potential treatment for individuals with active disease not responding to available therapies.

Safety

No serious adverse events have been reported in clinical studies of MSC for MS, excluding the above-mentioned case of transient encephalopathy with seizures in a subject receiving a dose equal to 100×10^6 MSC intrathecally.²⁷

The authors report that the subject was hospitalized and treated with valoprate and subsequently recovered completely. Other adverse events include some cases of meningeal irritation after intrathecal injection.^{25–27} After performing i.v. treatment, Connick et al. observed some minor adverse events such as a cutaneous rash a few hours after infusion in one patient, scalp pruritus in another, upper respiratory infection in one patient and one person with a urinary tract infection.²⁴ Overall, despite the limitations arising from the different clinical settings where MSC have been utilized, mainly in hemato-oncological conditions, the current experience with MSC in other diseases suggests that they are safe and well tolerated.

Efficacy of MSC treatment in MS

Only preliminary data are available from phase one open studies published so far. Promising preliminary data suggested some signal of efficacy on clinical parameters,²⁵ magnetic resonance imaging (MRI) measures²⁷ and immunological analysis.²⁵ However, the uncontrolled nature of the trials and the limited number of patients enrolled make these encouraging results yet anecdotal. Recently, the results of a clinical trial were published evaluating the efficacy of MSC in 10 patients with progressive MS with clinical evidence of optic nerve damage.²⁴ A linear mixed model was used to analyze clinical, electrophysiological and MRI data comparing variations in the pre-treatment year to the six-month post-treatment. Authors reported a limited impact of treatment on disability worsening, measured with the Expanded Disability Status Scale (EDSS); more important, authors observed an improvement in visual acuity correlating with an increase in optic nerve area on MRI and an increase in amplitude and latency of visual-evoked responses. As the authors themselves recommend, confirmation from larger studies is required but these results are of greatest relevance for the treatment of progressive MS.

Perspectives

On the basis of preclinical studies in EAE^{7,18–20,33} and of the encouraging safety data derived from clinical studies of MSC for other diseases, a group of experts in the field led by Antonio Uccelli (University of Genoa, Italy) and Mark Freedman (University of Ottawa, Canada) created, in 2009, the “International MSC transplantation (IMSC) study group” with the aim of designing a phase II clinical trial with MSC for the treatment of MS, to be conducted as a double-blind, randomized study with a cross-over design at different centers in Europe and North America.³⁸ According to the above considerations, a clinical protocol named “MEsenchymal StEm cells for Multiple Sclerosis (MESEMS)” using autologous MSC, at a dose of $1–2 \times 10^6$ /Kg of body weight to be injected intravenously, has been chosen. MSC will be administered in subjects with active

disease, including relapsing–remitting, secondary progressive with relapses and/or enhancing lesions, and primary progressive with enhancing lesions. After BM harvesting, MSC will be isolated, expanded under good manufacturing procedures (GMP) and cryopreserved until needed. Patients will be transplanted at baseline with MSC or placebo (unconditioned medium) and, six months later, subjected to another infusion, where subjects who had been treated with MSC will be treated with placebo and vice versa, in a double-blinded manner. Subjects will be followed clinically and with frequent MRI scans for 48 weeks after the first treatment to evaluate primary objectives, which will be safety of treatment and efficacy as per MRI parameters (gadolinium-enhancing lesions in MSC vs placebo treatment groups). Additional endpoints include cumulative MRI activity and brain atrophy, evidence of remyelination measured by magnetization transfer ratio (MTR), effect on clinical parameters, visual functions, neuropsychological tests and immunological responses. To circumvent financial constraints affecting such an academic trial, the study will be conducted as separate national studies following the same protocol and supported financially through local funding agencies. Data from single national trials will be collected through a single clinical record form (CRF), pooled in a unique data set and analyzed centrally in a blinded fashion in order to give numbers robust enough to allow powerful statistical analysis of safety and efficacy of MSC treatment. To date, ten MS centers in Europe have secured funds and obtained approval by national regulatory agencies and are ready to recruit patients. Several other centers in Canada and elsewhere are in the process of obtaining funds and will join this cooperative effort.

Conclusions

In the last decade, treatment of MS and other neurological diseases with stem cells has been regarded as the only way to achieve miraculous recovery from irreversible disability. This hype led to unrestrained sprouting of small studies and, dangerously, of uncontrolled treatments nurturing stem-cell tourism. Nevertheless, the current knowledge that has been achieved by robust preclinical studies with MSC has provided insights about their mechanisms of action and has highlighted the way to move ahead with a rigorous clinical approach. While available data do not support, at this stage, the possibility of regenerating the complex neural circuitry deranged by inflammatory events during the course of MS, current clinical trials will answer fundamental questions concerning the efficacy of MSC and will tell us whether these can play a role in the future armamentarium of treatments for MS, hopefully as one of the first steps toward tissue repair.

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Conflict of interest

The authors declare that they have no conflict of interest.

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