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Stem cell treatment in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic Lateral Sclerosis is a progressive fatal neurodegenerative disease that targets motor neurons. Its origin is unknown but a main role of reactive astrogliosis and microglia activation in the pathogenesis has been recently demonstrated. Surrounding neurons with healthy adjoining cells completely stops motor neuron death in some cases. Hence stem cell transplantation might represent a promising therapeutic strategy. In this study MSCs were isolated from bone marrow of 9 patients with definite ALS. Growth kinetics, immunophenotype, telomere length and karyotype were evaluated during *in vitro* expansion. No significant differences between donors or patients were observed. The patients received intraspinal injections of autologous MSCs at the thoracic level and monitored for 4 years. No significant acute or late side effects were evidenced. No modification of the spinal cord volume or other signs of abnormal cell proliferation were observed. Four patients show a significant slowing down of the linear decline of the forced vital capacity and of the ALS-FRS score. Our results seem to demonstrate that MSCs represent a good chance for stem cell cell-based therapy in ALS and that intraspinal injection of MSCs is safe also in the long term. A new phase 1 study is carried out to verify these data in a larger number of patients.

Keywords: Amyotrophic Lateral Sclerosis; Mesenchymal stem cells; Bone marrow; Transplantation; Spinal cord; Motor neuron diseases

1. Introduction

ALS is a devastating incurable neurodegenerative disease that targets motor neurons. It manifests as a linear decline in muscular function which eventually leads to paralysis, speech deficits and dysphagia. Within 2 to 5 years of clinical onset death, due to respiratory failure, occurs.[1] The vast majority of MND cases are sporadic; the aetiology of which is unknown and the pathogenesis is incompletely understood. Great interest has been focused on inflammatory processes and microglia activation. Microglia are subcomponents of the immune system that play a major role in advancing or limiting the disease [2]. Recent data have implicated the microenvironment of the motor neuron, rather than the motor neuron itself, as a primary target of the pathophysiology [3]. Some support for this hypothesis is provided by rodent models of ALS where focally delivered BM mononuclear cells modify the clinical phenotype [4,5]. In some cases, having normal cells completely stops motor neuron death [5]. In addition to immunomodulatory activity [5,6], several known properties of these cells, including production of neurotrophic factors [7] and stimulation of endogenous repair [8], may also be of relevance to their effect. Taken together these findings support a clinical translation trial of focally delivered cell therapies in human MND [6].

Mesenchymal stem cells from bone marrow are widely used in many human diseases because their potential to replicate as undifferentiated forms and to differentiate to multiple lineages [9] moreover their safety has been largely demonstrated [10]. Evidences of their differentiation in

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neural cells and glia have been reported by some authors both *in vitro* [11,12] and *in vivo* [13] but not confirmed by others [14,15]. Recently our laboratory studied human mesenchymal stem cells (hMSCs) in different cell culture conditions in both those already in use [11,12] and in new ones, from morphological, immunochemical, gene expression, and physiological points of view. In our new culture condition consisting in a neural progenitor maintenance medium (NPMM), containing growth factors such as hFGF, hEGF, NSF-1, hMSCs acquired new morphological characteristics, neural markers, and electrophysiological properties, which are suggestive of neural differentiation [16]. MSCs had shown the ability to promote neural recovery in different experimental models of neurological diseases [17,18]. MSCs selectively target injured tissue and promote functional recovery by different mechanisms such as the production of neurotrophic factors [19,20], the stimulation of angiogenesis and synaptogenesis [20], cell fusion [21]. The human mesenchymal stem cells when transplanted into the spinal cord of SOD 1 mice survive, proliferate and migrate and determine a significant reduction of the reactive gliosis and microglia activation resulting in the improvement of the behavioral tests [22]. This might lead to a clinical use of hMSCs in neural degenerative diseases. In this paper we present the preliminary results concerning the potential use of MSCs as therapeutic agents in ALS. Intraspinal delivery of the cells was needed because the impediment of stem cells to cross the blood brain barrier which is intact in ALS such as in other neurodegenerative diseases [23].

2. Methods

Inclusion criteria were: diagnosis of definite ALS according to the El Escorial criteria [24], mild to severe functional impairment at the spinal level, no or mild signs of bulbar involvement, no signs of respiratory failure, normal polysomnography, good understanding of the experimental protocol and normal psychological profile defined by Minnesota Multiphasic Personality Inventory (MMPI) test [25]. Patients were excluded if they had evidence of any concurrent illness or were receiving any medications that could affect bone. Standard therapies were used throughout the study.

The patients, in order to estimate the disease progression rate before transplantation, had a six months period of natural history observation. They were monitored every three months by clinical evaluation which included ALS-FRS [26], Norris score [27] and Forced Vital Capacity. These measures show a linear progressive decline during the course of the disease and are commonly used in clinical trials [28,29]. The Bulbar functions were also scored on a scale from 3 (normal) to 0 (markedly impaired) for speech and from 4 (normal) to 0 (markedly impaired) for eating, the maximum Bulbar score was 7 [30]. After MSC implantation the patients were monitored for at least 48 months by the same clinical assessment performed by the same examiners. Patients who became unable to attend the monitoring center were contacted by telephone: ALS-FRS scale [31] and an unstructured interview were delivered. Neuroradiological assessment consisted of MRI of brain and spinal cord before and after Gadolium DTA infusion. It was performed before transplantation and 1 week, 3, 6, 12, 36, 48 months after surgery. Somatosensory Evoked Potentials (SEPs) were also recorded before and after surgery. All patients were also examined at each visit by a clinical health psychologist and the following questionnaires were provided: Profile of Mood State(POEMS), [32] The SEIQoL-DW [33].

3. Experimental procedures

3.1. Bone marrow

Bone marrow was collected from the iliac crest in epidural anesthesia according to the standard procedure [34].

3.2. Isolation of MSCs

BM cells were centrifuged at 900 ×*g* for 15 min to discard the anticoagulant medium and then layered on a Percoll (Sigma Aldrich, St. Louis, MO, USA) gradient (density: 1.073 g/ml) and centrifuged at 1100 ×*g* for 30 min, according to a previously reported method [35]. The cells in the interphase were recuperated, washed twice with PBS1X (200 ×*g* for 10 min) and seeded at a density of 800,000/cm^2 in MSC Medium (Cambrex Bioscience, Versviers, Belgium) *containing* 10% of Fetal Bovine Serum (FBS) and maintained at 37 °C with an atmosphere of 5% CO_2. After 3 days, the nonadherent cells were removed and refeed every 3 to 4 days. In order to expand the isolated cells, the adhered monolayer was detached with trypsin/EDTA (Cambrex Bioscience, Versviers, Belgium) for 5 min at 37 °C, after 15 days for the first passage and every 7 days for successive passages. During *in vitro* passaging the cells were seeded at a density of 8000/cm^2 and expanded for 2 to 3 passages.

3.3. Cellular expansion analysis of MSCs

Cellular expansion growth rate of donor and patient MSCs was evaluated by cell count in a Burker Chamber at each passage and expressed in terms of population doubling (PD) as performed in the Stenderup et al. studies [36]. The immunophenotype analysis of adherent cells was performed, at each passage, by flow cytometry analysis. 2 to 5×10^5 cells were stained for 20 min with anti CD45 fluoroisothyocyanate (FITC), CD14 phycoerytrin (PE), CD90FITC, CD106PE, CD29FITC, CD44PE, CD105PE, CD166FITC (Becton Dickinson, San Jose, CA, USA) and 0.5 μg/ml of Propidium Iodide (Sigma) for the viability. Labeled cells were thoroughly washed with PBS 1X and were analyzed on a Epics XL cytometer (Beckman Coulter, CA, USA) with the XL2 software program. The percentage of positive cells was calculated using the cells stained with Ig FITC/PE as a negative control. At each passage MSCs were also analyzed for viability, sterility, mycoplasma detection, cytogenetic and telomeric analysis according to the guidelines of the Italian Institute of Health and showed by Mareschi et al [37].