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Autologous Mesenchymal Stem Cells for the Treatment of Amyotrophic Lateral Sclerosis

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ABSTRACT

Background: *Amyotrophic lateral sclerosis (ALS) is a motor neuron degenerative disease, causing progressive muscle weakness and death. Currently, there is no effective therapy to prevent its progression.*

Objectives: *The primary objective was to evaluate the safety and feasibility of autologous mesenchymal stem cell (MSC) transplantation in pre-rolandic motor and brain stem areas to treat ALS. A secondary objective was to evaluate its therapeutic efficacy through the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) and the functional status of the pyramidal tract using tractography neuroimages.*

Material and Methods: *A prospective, non-randomized study was conducted, including 12 ALS patients with autologous MSC transplantation between July 2011 and March 2013. Mean age was 57±11 years (29-67 years). Follow-up consisted of ALSFRS, tractography and a respiratory functional study at 6 and 12 months after MSC transplantation. The study assessed: a) the feasibility and safety of the procedure, b) survival and c) time until need for tracheostomy.*

Results: *Patients were divided into two subgroups; Group I: without manifest bulbar involvement and Group II: with evident bulbar involvement at the time of transplantation. Overall survival at one year post-transplantation was 58% (average survival: 8.8 months); in Group I 7/8 patients (86%) and in Group II, 1/4 patients (25%) survived (average survival: 12 and 4.6 months, respectively).*

Conclusions: *Neurotransplantation of autologous MSCs in ALS patients is feasible and safe. Patients with early or recently diagnosed disease, without manifest bulbar involvement would derive the greatest benefit, with better chance of survival and quality of life.*

Introduction

Amyotrophic lateral sclerosis (ALS) is a central nervous system degenerative disease in which superior motor neurons of the pre-rolandic motor area and inferior motor neurons of the anterior horn of the spinal cord gradually lose their function and die producing progressive muscular paralysis. Similarly, the disease may initiate in or affect in its progression the IX, X and XII cranial nerves, whose nuclei lie in the brain stem. Clinical manifestations are muscle atrophy in the topographic territory depending on the motor neurons of the anterior horn of the spinal cord, pyramidal tract abnormalities secondary to first superior motor neuron affected and functional involvement of bulbar innervated muscles.

This group of manifestations progressively worsen leading to a dismal prognosis with death due to respiratory insufficiency, dysphagia or the development of sudden arrhythmia within an average period of three years since the first manifestation of the disease. It affects mainly persons aged between 40 and 70 years, more frequently males and with higher incidence in people between 55 and 59 years of age. The prevalence is 2.6 to 3 cases per 100,000 persons, with an incidence of 2 cases per 100,000 persons per year (Table 1) [1-5]. A familial hereditary variant of ALS is also described, constituting only 5% of cases. In these patients, mutations in the genes encoding superoxide dismutase (SOD1) produce oxidative disorders by defective codification of the cytoplasmic Cu-Zn type 1 superoxide dismutase. The most common genes involved in ALS are C9orf72, SOD1, TARDBP and FUS. According to the meta-analysis performed by Zou et al. in 2016 in Asian and European populations, these genes are globally mutated in 47.7% of familial ALS and in 5.2% of sporadic cases. In the European populations the most common mutation was C9orf72 repetition (33.7% in familial and 5.1% in sporadic ALS), followed by SOD1 mutation (14.8% in familial and 1.2% in sporadic ALS), TARDBO (4.2% in familial and 0.8% in sporadic ALS) and FUS (2.8% in familial and 0.3% in sporadic ALS). However, in Asian populations, the most frequent mutations were SOD1 followed by FUS; C9orf72 and TARDBP. As this disease manifests in Latin American populations, it is under investigation through a project carried out by the Latin American Epidemiologic Network for ALS (LAENALS) and its results are expected to be finished by 2021 [6].

No solid evidence has been found so far supporting a relationship between environmental factors and ALS. However, using inverse variance, the Karolinska Institutet study (with data of the MinE project) seems to have found a statistically significant causal association between smoking habit and the risk of ALS (odds ratio: 1.25, 95% CI: 1.01-1.55, p=0.04), though more exhaustive studies are necessary [6]. Some studies report an association between high plasma concentrations of persistent organic pollutants, with a negative impact on survival of ALS patients [7].

Although genetic causes provoke sporadic cases, most cases have been ascribed to glutamate-mediated excitotoxicity processes, oxidative stress, mitochondrial damage, cytoskeletal and axoplasmic transport abnormalities, autoimmunity phenomena, infective factors such as viral infection, and trophic and exogenous toxic factors as possible responsible causes of its pathophysiological origin [8].

There is currently no effective treatment capable of preventing the progress of this entity. The only drug available, accepted by the US Food and Drug Administration is riluzole, which has been shown to prolong the life of patients, reducing the risk of death by 35% at 12 and 18 months of its administration but without quality of life improvement. Riluzole acts by blocking glutamate release, an excitatory neurotransmitter postulated in the excitotoxic theory as responsible for triggering neuronal degeneration.

Considering that ALS is a chronic progressive disease leading to major disability and death and for which we have currently no therapeutic modifiers of its morbid course, we postulate autologous mesenchymal stem cell (MSC) transplantation to modify the neuronal environment in which the process of programmed death (apoptosis) takes place and hence prevent the natural course of this progressive degeneration [9-11]. The rationale for this hypothesis finds support in the institution's ample experience with stem cells for the treatment of heart diseases [12].

Objective

The aim of the present study was to evaluate the safety and feasibility of the treatment with autologous MSC transplantation in motor pre-rolandic areas and at the brain stem level. The secondary objective was to determine the use of appropriate tools to assess the therapeutic efficacy of the procedure by means of clinical follow-up using the Amyotrophic Lateral Sclerosis Functiomal Rating Scale FRS (ALSFRS) and the functional status of the pyramidal tract through tractography with functional magnetic resonance imaging studies.

Material and Methods

A prospective, non-randomized study was conducted in patients attending the Neurology Clinic of Hospital Presidente Perón. Twelve patients (10 male and 2 female) with certified clinical diagnosis of ALS according to Escorial clinical criteria (1990) were transplanted with autologous MSCs between July 2011 and March 2013. Mean age was 57 ± 11 years (29-67 years). Inclusion criteria consisted in accepting only patients with clinically certified ALS, with optimal conventional medical treatment and who had previously signed the informed consent. Selected patients underwent a complete clinical evaluation and electromyography study confirming peripheral motor neuron involvement, ruling out in all cases the possibility of multifocal motor neuropathy due to conduction block. The evaluation prior to the transplantation procedure included: 1) ALSFRS evaluation and 2) tractography neuroimages derived from a functional magnetic resonance imaging study with diffusion tensor imaging (DTI) technique, which is sensitive to the movement of water molecules in a tissue and reveals the in vivo integrity and orientation of the white matter nervous tracts (Figure 1) [13]. Clinical follow-up was performed through ALSFRS, tractography and a respiratory functional study at 6 and 12 months after autologous MSC transplantation, which was the only therapeutic procedure added to the conventional treatments that remained without modifications. The study assessed: a) the feasibility and safety of the procedure, b) survival, defined as life time until death and c) time elapsed until need for tracheostomy.

A special legal authorization was granted through a writ of amparo presented before the authorities of the Ministry of Health of the Province of Buenos Aires and all the patients signed an informed consent especially designed for neurotransplantation of autologous bone marrow mesenchymal cells and puncture of the iliac crest to harvest them.

Autologous Mesenchymal Stem Cell Harvesting, Processing and Transplantation

Between 20 to 40 days prior to the transplantation procedure bone marrow (BM) was harvested by iliac crest puncture and BM aspiration. The procedure was performed in the operating room under local anesthesia, using Harvest 11 G × 10 cm aspiration needles with side openings to draw a BM volume of 15-200 ml. The collected sample was placed in a container with RPMI culture media for later laboratory processing in a laminar flow hood. Sample analysis, culture and processing was done in the laboratory following Good Manufacturing Practice (GMP) guidelines. Isolation and culture of mesenchymal cells was performed under biosafety measures in gassed incubators. The approximate processing time was 60 days, with periodical weekly evaluations. The quantification of the final product cellularity to transplant was completed with flow cytometry.

Endovascular MSC transplantation was performed via super-selective micro catheterization of intra-cerebral arteries visualized with high-resolution digital subtraction angiography. Continuous brain stem functional monitoring during the transplantation procedure was done through early brain stem auditory evoked potentials [14,15]. Currently, technological advances allow low-radiation, high-resolution digital cerebral angiography, with visualization of secondary and third order arterial branches. Neuronavigation through the combined use of low-profile, totally atraumatic micro-catheters and micro-needles (0.010 and 0.014 inches) and flow-directed micro-catheters (1.2 inches) with specific imaging techniques, provides road mapping with information of the path to follow in the complex intracranial circulation. During the MSC transplantation procedure, micro-catheters were inserted in left and right pre-rolandic arteries, and via the posteroinferior cerebellar arteries the low brain stem area (bulbar) was accessed through the bulbar lateral sulcus artery. Transplantation of the MSC suspension was performed by superselective 5 cc pulse infusions of the cellular preparation, both in the left and right pre-rolandic areas and in the bulbar area.

The patient remained under neuroleptoanalgesia during the course of the procedure, with close monitoring of vital signs and anticoagulated with heparin at a dose of 50-70 IU/kg. Once the procedure ended, all the micro-systems were withdrawn and specific angiographic controls were carried out to observe all the intracranial arteries. Then, the patient was transferred to the intensive care unit to monitor and control his evolution for 24 h. In all cases, the procedure was well tolerated, without complications associated to the iliac crest puncture or the neuronavigation technique. Only 2 patients reported headaches which subsided with analgesia. All patients were discharged on the second day post-transplantation.

Results

A total of 12 autologous MSC transplantation procedures were performed, 7 (58%) of which presented with bulbar involvement prior to the procedure and among this group, 4 had evident signs of bulbar involvement with manifest dysphagia, dysarthria and respiratory disorders. A 29-year old patient started with symptoms after a surgical procedure which required general anesthesia (exogenous toxic?).

Patients were divided into two subgroups; Group I: without manifest bulbar involvement and Group II: with evident bulbar involvement at the time of transplantation.

Overall survival at one year after transplantation was 58%. In Group I survival was 7/8 patients (86%) and in Group II, 1/4 patients (25%). This patient survived for 3 years but required respiratory assistance (tracheostomy) at one-year post-transplantation. Group I average survival was over 12 months, while that of Group II was 4.6 months, with overall survival for both groups of 8.8 months. It is probable that in the Group II patients, the short time between transplantation and death did not allow the procedure to achieve a therapeutic effect.

The study results characterize two well-defined groups of patients: Group I, without severe bulbar involvement and better perspective of response to treatment. When the bulbar involvement is mild or of recent onset, these patients are amenable to treatment with better chance of survival and even quality of life improvement. From this group we followed-up 2 patients who showed recovery or stabilization of their functional clinical scale, one with a 9-point improvement and the other with only a drop of one point at one year after the procedure (Figures 2 and 3).

Group II of high-risk patients with evident bulbar involvement did not obtain any benefit with MSC therapy and, therefore, should not be considered for MSC transplantation.

Discussion

Gradual and progressive motor neuron loss occurring in the pre-rolandic motor areas, in the cervical, dorsal and lumbar segments of the anterior horns of the spinal cord and at the level of the nuclei of the bulbar cranial nerves produce extreme muscular weakness affecting swallowing, speech, respiratory function and segmental muscle force. The therapeutic goal of in-situ autologous mesenchymal cell grafting in the vicinity of these topographic areas is associated with multiple restorative mechanisms, including cellular fusion, synthesis of trophic factors, the stimulus for endogenous stem cell proliferation, and trans differentiation phenomena that might explain possible results attributable to the niche formed by local stem cell implants.

Srivastava [14] already posed in 2008 that the dysfunction of certain glial cell populations, holding and supporting motor neurons, could be responsible for initiating the morbid process leading to degenerative neuronal apoptosis. We have referred to the leading role attributed to excess of excitatory neurotransmitters, mainly glutamate. Failure of its transporters would create the possibility of abnormal glutamate accumulation in the extracellular space, responsible for initiating a chain of metabolic reactions that

involve the onset of the degenerative process of programmed cell death known as apoptosis. Glutamate in the extracellular space is incorporated into the astrocytes by EAAT1 and EAAT2 transporters and once inside the cell is transformed into glutamine by the enzymatic action of glutamine synthetase. Functional failure of the glutamate transporter would produce a toxic excitation leading to neuronal death.

The modifications that the *in-situ* stem cell transplantation could eventually develop in the environment where these neuronal deleterious changes take place would provide rational support for their use. NT3 neurotrophic factor, neurotrophic growth factor (NGF), and glial neurotrophic factor (GNF) secretion have been able to demonstrate their neuroprotective effect in transgenic rats [16].

Garbuzova y col [17] showed in 2003 the beneficial effect of the NT2 cell line from human stem cells transplanted into transgenic mice with motor neuron injury. Several studies have employed stem cells for this disease, using different transplantation pathways, either intravenous, intrathecal or through surgical access of the spinal cord [18-20].

Conclusion

Our project included stem cell transplantation in both pre-rolandic areas and in the brain stem via a selective endovascular catheterization method with minimal anesthetic requirements and lower risk of complications than a neurosurgical intervention. We consider that patients with early or recently diagnosed disease, without manifest bulbar involvement would represent the group with greater impact to benefit from this type of rational therapy.

Neurotransplantation of MSCs by selective endovascular micro-catheterization of pre-rolandic and brain stem areas was feasible and safe in our ALS patients. We detected no intraprocedural brain stem involvement. Follow-up observation with ALSFRS and functional pyramidal tract status with magnetic resonance DTI studies, allow us to conclude that neurotransplantation of autologous MSCs would be feasible in patients without severe brain stem functional involvement, with better chance of survival and quality of life.

Table 1: Worldwide incidence and prevalence of amyotrophic lateral sclerosis.

Countries	Incidence (10^5 persons/year)	Prevalence (10^5 persons/year)
Argentina	3.17	8.86
Canada	2.24	NA
Europe	2.08	5.4
USA	1.75	3.4
Japan	1.97	11.3
China	0.46	2.01
Uruguay	1.37	1.9
Costa Rica	0.97	NA
Brazil	0.4	0.9 to 1.5
Ecuador	0.2 to 0.6	NA

NA: Not available.

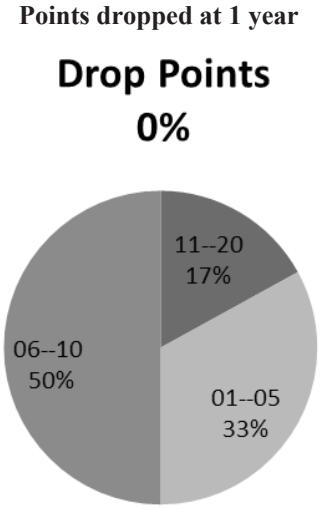

Figure 1: Functional magnetic resonance imaging allows pyramidal tract assessment of fiber density at various topographic levels in their descent to the spinal cord.

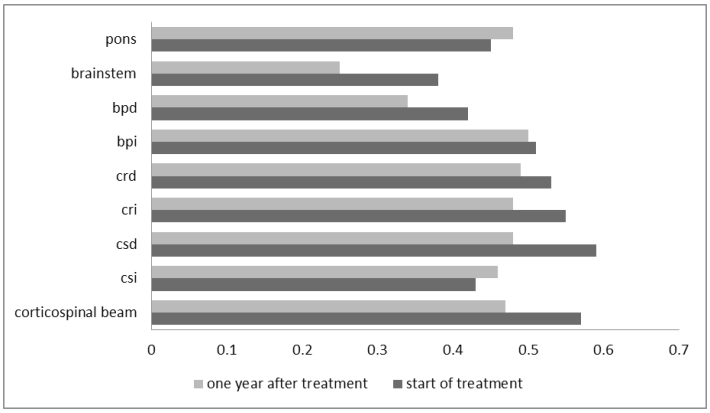

Figure 2: Clinical severity assessment with ALSFRS at one year of disease progression.
Different values obtained with tractography in the same patient at the beginning and end of treatment.

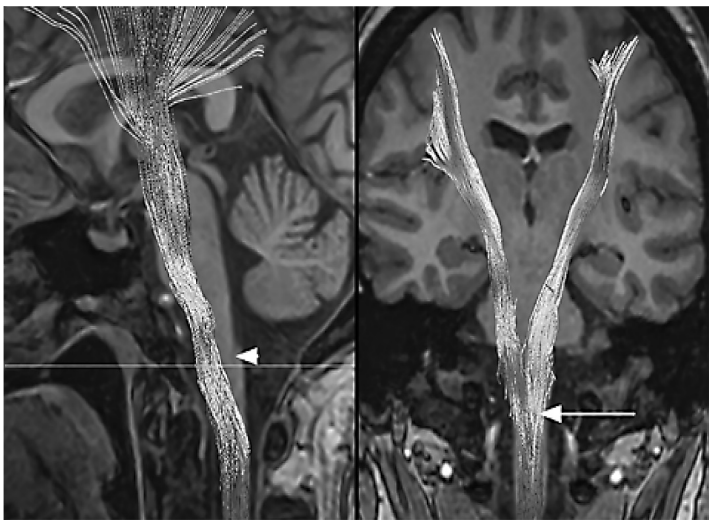

Figure 3: Evolution control tractography allows quantification of axon loss in different topographic sectors of the pyramidal tract.

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