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Breathing pattern in a phase I clinical trial of intraspinal injection of autologous bone marrow mononuclear cells in patients with amyotrophic lateral sclerosis

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ABSTRACT

The safety of autologous bone marrow mononuclear cells (ABMNC) intraspinal infusion in amyotrophic lateral sclerosis (ALS) patients was evaluated considering breathing and sleep patterns. Patients between 20 and 65 years old were eligible if they had definite ALS, spinal onset, a disease duration between 6 and 36 months, FVC > 50%, and a below 90% oxygen saturation (T90) <2% of sleep time. The transplant was performed 6 months after enrollment. ABMNC were infused at thoracic 3–4 level. Eleven patients were included. The REM sleep decreased slightly one year after the cell transplant but not significantly. There were no differences in apnea–hipopnea index, mean oxygen saturation and nadir desaturation evolution. An increase of T90 was observed 180 and 360 days after injection (2.95 ± 1.51% and 4.30 ± 4.10% respectively), although it was not statistically significant. The central drive determined by occlusion pressure (P01) and inspiratory flow showed non-significant differences after one year. Intramedullary injection of ABMNC did not worsen the cortico medullar diaphragmatic pathways.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unknown pathogenesis that leads to motor neuron apoptosis; the patients develop progressive muscular atrophy that leads them to death. Respiratory failure is the main cause of death, which takes place at a mean of 3 years after a diagnosis (Talbot, 2009).

Lung function is an important prognostic factor in this disease due to the involvement of respiratory muscles and lower motor neuron. It has been shown in large retrospective studies that a high Forced Vital Capacity (FVC) predicts a better survival rate (Czaplinski et al., 2006). However, there is data suggesting that the diaphragm motor cortex may be affected even before the symptoms start (Nichols et al., 2013; Miscio et al., 2006). Moreover, patients with normal lung function and preserved diaphragmatic function have desaturation episodes during sleep which could be related to central drive dysfunction (Atalaia et al., 2007). Recently, it has been found that some ALS patients who have excellent diaphragmatic EMG activity and lower motor neuron innervation of the diaphragm present centrally mediated respiratory instability (Onders et al., 2015). It is also known that diaphragmatic dysfunction – clinically detected either by cervical or transcranial magnetic stimulation – is present in ALS patients with sleep disorders as, for example, REM sleep reduction (Arnuff et al., 2000). In fact, central drive and REM sleep reduction can be considered as an initial signs of respiratory control disfunction involvement.

We have published a pilot safety study that confirmed the safety of intraspinal infusion of autologous bone marrow mononuclear

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cells in ALS patients and provided evidence about their neurotrophic activity (Blanquer et al., 2012). The primary outcomes assessed were neurological scales ALS-FRS, Norris, MRC and lung functional parameters as FVC, MIP and MEP. In the present study, we also assess secondary outcomes like the breathing pattern and sleep parameters to evaluate the functionality of the cortical diaphragmatic pathways.

2. Material and methods

We designed an open single arm phase I trial to evaluate the feasibility and safety of intraspinal infusion of autologous BMNC. Safety was defined primarily as the absence of treatment-related serious adverse events, as defined by the CONSORT group (Ioannidis et al., 2004), and, secondarily, as a decrease rate in the forced vital capacity (FVC), the ALS-functional rating scale (FRS), Norris, and the MRC scales not significantly higher than before the therapy. In order to detect cortical diaphragmatic pathway dysfunction we evaluated, as secondary outcomes, the rate of sleep efficiency, percentage of REM sleep, the increase rate of occlusion pressure p01 and the TV/Ti ratio as an estimation of the central respiratory drive. P01/MIP ratio was also measured as an index of central respiratory output in relation to the inspiratory muscle strength.

The selection criteria have been previously published (Blanquer et al., 2012). Briefly, patients between 20 and 65 years old were eligible when diagnosed with definite ALS according to the El Escorial criteria (Brooks, 1994; Brooks et al., 2000), spinal onset, and duration of disease between 6 and 36 months. FVC had to be >50% of predicted, and sleep time oxygen saturation below 90% (T90) $\leq$2%. Patients were excluded if they had evidence of concomitant neurological, psychiatric or systemic disease, if they had received treatment with corticosteroids, immunoglobulins, or immunosuppressive drugs in the last 12 months, or if they had been included in another clinical trial, required enteral or parenteral nutrition, were pregnant or were unable to provide informed consent.

We were able to recruit 13 patients after an intensive selection process that included a complete medical, respiratory, and neurophysiological assessment, which was performed at the initial visit. A psychological evaluation was carried out to ensure that the patients fully understood the experimental nature of the trial and the risks associated with the procedure as well as to assess their psychological stability. Finally, 11 patients were included and two patients were excluded because during the quarterly evaluation they did not meet the inclusion criteria due to disease progression.

The transplant was performed 6 months after enrollment. After laminectomy, ABMNC were infused intramedullary at thoracic 3–4 level to ensure spinal column stability and safety of the procedure. The transplant procedure has been described in detail elsewhere (Blanquer et al., 2010). After the infusion, all patients were assessed by neurologists at two different hospitals quarterly for 1 year. In addition, in order to further monitor the adverse events, open telephone interviews were performed weekly for 3 months and monthly thereafter, until the end of the 1 year follow-up period. After the end of the follow-up non periodical telephone interviews were performed, the last being the 7th of September of 2015. Adverse events were graded according to the common terminology criteria for adverse events v3.0 (CTCAE) (Trotti et al., 2003). The clinical trial was approved by the Clinical Trials Ethics Committee of the Virgen de la Arrixaca University Hospital and the Morales Meseguer University Hospital as well as by the Agencia Española de Medicamentos y Productos Sanitarios. AAI Pharma (Madrid, Spain) acted as the external monitor of the study. The trial was registered at www.clinicaltrials.gov (identifier NCT00855400) and at the European Clinical Trials Database (EudraCT number 2006-003096-12).

2.1. Assessments

Polysomnography and breathing pattern were performed quarterly from day −180 to +360, day 0 being the transplant date. Spirometry and respiratory muscle assessments were performed with a ZAN 100 pulmonary spirometer and a ZAN 500 Plethysmograph (Waldfenster, Germany, www.zan.de). FVC was measured in the sitting position and was expressed as a percentage of the predicted value. Static mouth pressure was measured using a flanged mouthpiece. Patients performed three maximal inspiratory and expiratory efforts from residual volume or total lung capacity, and then maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) measurements were recorded (Polkey et al., 1995). Polysomnography was performed following the AASM criteria (Iber et al., 2007) with a 20-channel Neurofax Nihon-Kohden (Nihon Kohden, Tokyo, Japan, www.nihonkohden.com).

2.2. Breathing pattern

While in a sitting position and after 10 min of rest, the patient was instructed to breathe through the "mouthpiece" (Burski, 1989). After the patient had developed an "steady state" period in his breathing pattern for at least 2 min, minute ventilation (VE), breathing pattern (tidal volume (TV), respiratory frequency (RF), duty cycle (iT/totT) and mean inspiratory flow (TV/iT) were recorded and assessed by the flow signal. Respiratory drive (P01) was calculated as the change in pressure occurring between 100 ms prior to airflow start and the onset of flow from P01. TV/iT respiratory impedance was also calculated (P01/TV/iT). The final P01 and TV/iT were calculated as a mean of six occlusion procedures (Whitelaw et al., 1975).

2.3. Statistical analysis

Continuous variables were described using central tendency measures (median or mean) and standard error. The conditions of application of statistical analysis were checked and normality was verified by the Kolmogorov–Smirnov test and homoscedasticity by the Levene test. Comparison of continuous variables was carried out using paired Student's t test and the repeated measures ANOVA test (independent variable: time at which subjects are tested). Significant results from ANOVA tests were followed up by post hoc pair-wise comparisons using Bonferroni test. Two different analyzes were performed until day 180 visit and until day 360 visit in order not to lose information. If variables did not follow normality criteria according to the Kolmogorov–Smirnov test, the non-parametric Kruskal–Wallis test was applied. The analysis was performed by comparing the variables at 6 and 12 months. The level of significance in all statistical tests was alpha $\leq$0.05.

3. Results

Eleven (five males, six females) patients were included in the trial with a median age of 46 (mean 46.81 ± 2.35, range 32–61) years. The median duration of the disease from diagnosis to the ABMNC infusion was 21 months (mean 22.81 ± 2.73, range 15–40). The median FVC at entry was 105% (mean 106.27 ± 3.76, range 79–120), the median of maximal inspiratory pressure MIP was 7.54 KPa (mean 7.03 ± 0.91, range 1.54–12.28) and the median of maximal expiratory pressure was 7.34 KPa (mean 7.43 ± 0.72, range 3.97–10.26). The median ALS-FRS, Norris and MRC scores were 30 (30.83 ± 1.21, range 24–38), 74 range (75.91 ± 2.97, range 54–95) and 46 (44.66 ± 1.64, range 35–54). The details of these variables and progression for each patient have been previously published (Blanquer et al., 2012). All patients received their usual medical treatment, including Riluzole.