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Intrathecal administration of autologous mesenchymal stromal cells for spinal cord injury: Safety and efficacy of the 100/3 guideline

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Abstract

Background aims. Cell therapy with autologous mesenchymal stromal cells (MSCs) in patients with spinal cord injury (SCI) is beginning, and the search for its better clinical application is an urgent need. *Methods.* We present a phase 2 clinical trial in patients with chronic SCI who received three intrathecal administrations of 100 x 10^6 MSCs and were followed for 10 months from the first administration. Efficacy analysis was performed on nine patients, and safety analysis was performed on 11 patients. Clinical scales, urodynamic, neurophysiological and neuroimaging studies were performed previous to treatment and at the end of the follow-up. *Results.* The treatment was well-tolerated, without any adverse event related to MSC administration. Patients showed variable clinical improvement in sensitivity, motor power, spasms, spasticity, neuropathic pain, sexual function or sphincter dysfunction, regardless of the level or degree of injury, age or time elapsed from the SCI. In the course of follow-up three patients, initially classified as ASIA A, B and C, changed to ASIA B, C and D, respectively. In urodynamic studies, at the end of follow-up, 66.6% of the patients showed decrease in postmicturition residue and improvement in bladder compliance. At this time, neurophysiological studies showed that 55.5% of patients improved in somatosensory or motor-evoked potentials, and that 44.4% of patients improved in voluntary muscle contraction together with infralesional active muscle reinnervation. *Conclusions.* The present guideline for cell therapy is safe and shows efficacy in patients with SCI, mainly in recovery of sphincter dysfunction, neuropathic pain and sensitivity.

Key Words: *cell therapy, mesenchymal stromal cells, neuropathic pain, sphincter dysfunction, spinal cord injury*

Introduction

At present, cell therapy using autologous mesenchymal stromal cells (MSCs) is configured as a hope to improve the quality of life in patients with spinal cord injury (SCI) [1–12]. However, these new techniques are still in their infancy and it is necessary to know many details, such as the criteria to select the patients that can have better benefit, or to know dosage or administration guidelines. Based on previous experiences in animal models and in humans, intrathecal or intralesional administration of MSCs are a safe and useful strategy to achieve benefit in SCI, with the condition that the spinal cord is not anatomically sectioned [11,12], which implies a careful study with magnetic resonance imaging (MRI) of each SCI [13]. In this same line of knowledge, our previous experience suggests that autologous MSCs are superior to the use of allogeneic MSCs, and that use of autologous plasma as a support for MSCs is better than saline [11]. On the other hand, previous studies in patients suggest that the benefit of this type of cell therapy is not related to the greater or lesser time of chronicity of the SCI. Furthermore, at least in certain efficacy parameters, there seems to exist a relationship between the benefit and the number of cells administered [11].

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To obtain more experience in the possibilities of different guidelines of MSC administration in patients with SCI, here we present the results of a phase 2 clinical trial that analyzes the efficacy and safety of intrathecal administration of autologous MSCs with a guideline of three doses of 100 million MSCs, with an interval of 3 months between each administration (ClinicalTrials.gov NCT02570932, EudraCT: 2014-005613-24, Spanish Agency of Medicament and Health Products (AEMPS) number: 15–0253).

Methods

Cell therapy medicament

We used the NC1 medicament. It is a cell therapy medicament developed after pre-clinical studies by our group and currently approved as a medicament under clinical investigation (PEI number: 12–141) by the AEMPS. The medicament consists of autologous MSCs and autologous plasma as its excipient. Previous to NC1 preparation, a sample of peripheral blood was retrieved from each patient for genomic studies to rule out chromosomal abnormalities that could discourage cell expansion, and to obtain a genetic fingerprint (KaryoNIM Stem Cells and KaryoNIM STR test, respectively; NIMGenetics).

The genetic studies included the following: (1) analysis of the cellular genome by techniques Array comparative genomic hybridization (CGH) to ensure that the starting cells are genetically stable and the expansion process produces no genetic modification, and (2) analysis of genetic fingerprinting (Short Tandem Repeat [STR]) with the aim of having no cross-contamination with other cells in the manufacturing process. The genetic fingerprint analysis was done by the external laboratory NIMGenetics, with a panel of 20 markers + amelogenin. The panel was amplified by multiplex polymerase chain reaction (PCR) using the PCR kits PlusTM AmpFlSTR Identifiler Amplification Kit and/or PCR NGM AmpFlSTR Amplification Kit (NIMGenetics). The analysis by capillary electrophoresis of the amplified products was performed on an ABI automated sequencer 3100 - Avant.

For the analysis of the profiles obtained, Gene Mapper software v 3.2.1 was used. The analysis of possible genetic abnormality was performed using a platform CGH Array (KaryoNIM STEM, NIMGenetics) optimized for use in cell therapy projects. This platform consists of 60 000 probes distributed throughout the genome with a probe designed for each 60 kb and enables efficient detection of amplifications and deletions >200 Kb and analysis in detail of 407 genes related to genomic instability and abnormal proliferation in accord with the Cancer Gene Census list. It has an enrichment probe designed specifically for the detection of 395 cancer-related genes, included in the Cancer Gene Census list (genes for which have been described causal mutations involved in cancer) with an average of five probes per gene. The platform includes 15 specific probes for detecting each of the 23 oncogenes commonly used in clinical diagnosis. In addition, there are probes designed specifically for the detection of six genes associated with stem cells. The minimum degree of mosaicism detected by array CGH is 20%–30%. The scanning process allows the use of arrays 8 x 60 k with a resolution of 2 μm.

For obtaining the excipient, as a first step in the preparation of the NC1, we start with the removal of 500 mL of peripheral blood from each patient. In our cleanroom, blood was centrifuged at 900*g* for 8 min to obtain the plasma fraction, which is aliquoted in 15-mL tubes and stored at -80°C until the medicament formulation.

Approximately 2 weeks later, 50 mL of bone marrow was aspirated under aseptic conditions from the iliac bones of each patient, immediately anticoagulated by a 5 mL solution composed of 100 IU/mL sodium heparin Chiesi (ChiesiEspaña, L'Hospitalet de Llobregat, Spain) and 10^4 IU/10^4 μg penicillin-streptomycin (BioWhittaker-Lonza) and sent to our cleanroom for culture and expansion under good manufacturing practice (GMP).

Mononuclear cells (MNCs) were separated by density gradient, using an automated cellprocessing system (SEPAX; BioSafe). Then, they were plated at a density of 16 x 10^4 to 20 x 10^4 cells/cm^2, in 175-cm^2 flasks on Alpha-Minimum Essential Medium (MEM) with Earle's Balanced Salt Solution (BSS), and supplemented with 20% prion-free LGC standard serum (SLU ATCC.SCRR-3020, lot 63753841; Salvador Spriu) and 10^4 IU/10^4 μg penicillin-streptomycin (BioWhittaker-Lonza). The cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere for 3 days, after which nonadherent cells were removed by replacing the medium. When the cultures approached confluence (90%–100%), adherent cells were detached by treatment with trypsin/ethylenediaminetetraacetic acid (EDTA) solution (BioWhittaker-Lonza). Neutralization of trypsin and subsequent washing were performed with Alpha-MEM medium supplemented with 10% fetal bovine serum (FBS) and 2 mmol/L L-glutamine, centrifuging at 1250 rpm for 10 min. After study of viability, cells were cultured to obtain the required number according to the plan previously made for each patient. Cells were replated at a density of 3000–5000 cells/cm^2 in factory farming of four floors with free-antibiotic Alpha-MEM medium supplemented with 10% FBS and 2 mmol/L L-glutamine, and the culture was maintained renewing the medium every 3–4 days until there was a confluence of 90%–100%. Once the