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Research paper

Intrathecal administration of autologous bone marrow stromal cells improves neuropathic pain in patients with spinal cord injury

J. Vaquero*, M. Zurita, M.A. Rico, C. Aguayo, C. Fernández, R. Gutiérrez, G. Rodríguez-Boto, A. Saab, R. Hassan, C. Ortega

Service of Neurosurgery, Puerta de Hierro-Majadahonda Hospital, Autonomous University, Madrd, Spain

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ABSTRACT

Neuropathic pain (NP) is highly disabling, responds poorly to pharmacological treatment, and represents a significant cause of decreased quality of life in patients suffering from spinal cord injury (SCI). In recent years, cell therapy with autologous mesenchymal stromal cells (MSCs) has been considered as a potential therapeutic weapon in this entity. Ten patients suffering chronic SCI received 100 million MSCs into subarachnoid space by lumbar puncture (month 1 of the study) and this procedure was repeated at months 4 and 7 until reaching a total doses of 300 million MSCs. Intensity of NP was measured by standard numerical rating scale (VAS) from 0 to 10, recording scores previous to the first MSCs administration and monthly, until month 10 of follow-up. Months 1, 4, 7 and 10 of the study were selected as time points in order to a statistical analysis by the nonparametric Wilcoxon rank test. Our results showed significant and progressive improvement in NP intensity after the first administration of MSCs (p: 0.003). This study supports the benefit of intrathecal administration of autologous MSCs for the treatment of NP in patients with SCI.

1. Introduction

Neuropathic pain represents one of the major neurological sequelae in patients with spinal cord injury (SCI) and it is difficult to treat, despite currently available pharmacological and neuromodulation advances [1]. In recent years, some experimental studies in rodents [2,3,4,6,12] have drawn attention to its treatment by cell therapy techniques, particularly with the use of mesenchymal stem cells (MSCs), possibly through the release of Transforming Growth Factor-beta-1 (TGF-β1) secretion by transplanted cells [2].

On the other hand, the use of MSCs as a treatment for sequelae of SCI in humans seems to open a door of hope in these patients, improving their quality of life. Recently has been reported that cell therapy in patients with SCI can achieve recovery in sensitivity and sphincter control, and particularly in neurogenic bowel dysfunction [8,9]. In these early experiences, data have been collected in favor of some paraplegic patients are able to improve neuropathic pain in the course of treatment, although the small number of patients with neuropathic pain in these clinical trials has prevented conclusions with statistical significance [9].

In the present study we analyzed the benefit obtained on NP by the intrathecal administration of autologous MSCs in a group of 10 patients suffering chronic SCI with NP.

2. Materials and methods

2.1. Data of patients

Ten patients (M/F: 9/1) with chronic SCI and symptoms of NP were studied. Seven of them were included in the ClinicalTrials.gov: NCT02570932, designed to study the benefit of three doses of 100 million MSCs by subarachnoid route in patients with chronic neurological dysfunction due to SCI. Other three patients with chronic SCI and neuropathic pain received the same treatment by compassionate use, after the approval of the Spanish Agency for Medicament (AEMPS) and our Medical Management.

The patients had a pre-treatment age between 26 and 62 years (mean ± SD: 45.10 ± 10.62 years). The time from SCI to the start of cell therapy ranged from 3 to 44 years (18.10 ± 16.67 years). SCI was located at cervical region in 5 patients, at thoracic level in 2 and at lumbar region in 3 patients. In the assessment of the American Spinal Injury Association (ASIA) [5]. Three patients were ASIA A, two patients ASIA B, three patients ASIA C and two patients were ASIA D. At the start of treatment none of the patients had a maintained regimen of analgesia because of neuropathic pain. They were medicated irregularly, without an established pattern and were instructed not to change these habits during the treatment of cell therapy. The measure of NP

* Corresponding author at: Puerta de Hierro Hospital, Neurosurgical Service, Manuel de Falla, 1. 28222-Majadahonda, Madrid, Spain.
E-mail address: jvaqueroc@telefonica.net (J. Vaquero).

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was performed by standard numerical rating scale from 0 to 10 (VAS: Visual Analog Scale) [11].

2.2. Treatment

In all cases, the patients received in the subarachnoid space, by lumbar punction a total of 300 million of our cell therapy medicament (NC1). The first administration (100 million) was performed at month 1 (basal time), and it was repeated at months 4, and 7 of follow-up. Evaluation of NP was performed prior to the first administration of MSCs and was repeated monthly until the final evaluation, performed 10 months after the start of treatment.

2.3. Cell therapy medicament

NC1 is a cell therapy medicament, developed by our group and currently approved as a medicament under clinical investigation by the AEMPS (PEI No. 12–141). The medicament consists of autologous MSCs and autologous plasma as its excipient [8]. Previous to NC1 preparation, a sample of peripheral blood was retrieved from each patient for genomic studies in order 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, Madrid, Spain).

For culture and expansion of MSCs, approximately 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 by 100 IU/ml sodium heparin Chiesi (ChiesiEspaña, L'Hospitalet de Llobregat, Spain) and 10^4 IU/10^4 µg penicillin-streptomycin (BioWhittaker-Lonza, Madrid, Spain) and sent to our cleanroom for culture and expansion under good manufacturing practice (GMP).

Mononuclear cells (MNC) were separated by density gradient, using an automated cell-processing system (SEPAX, BioSafe, Madrid, Spain). Then, they were plated at a density of 16×10^4 to 20×10^4 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% american prion-free fetal bovine serum (FBS) (LGC standard serum (SLU ATCC-SCR*R*-302020, lot 63753841, Salvador Spriu, Barcelona, Spain), and 10^4 IU/10^4 µg penicillin-streptomycin (BioWhittaker-Lonza). The cultures were maintained at 37 °C in a humedified 5% CO_2 atmosphera 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 was performed with Alpha-MEM medium supplemented with 10% FBS and 2 mM 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 4 floors with free-antibiotic Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, and the culture was maintained renewing the medium every 3–4 days until a confluence of 90–100%. Once the culture reached confluency, it was prepared to obtain the bulk of MSCs. At this time, MSCs were detached with trypsin/EDTA and washed with Hank's BSS medium (BioWhittaker-Lonza) supplemented with 5% albumin (20% albumin Grifols, Barcelona, Spain). After it, MSCs were resuspended with the previously obtained autologous plasma in order to remove traces of the washing medium. After cell counting, MSCs for the successive doses were separated and then cryopreserved, at a concentration of 2.2×10^6 cells/ml in a FBS solution, in dimethylsulphoxide (DMSO) (Miltenyi Biotec, Madrid, Spain). For it, we used a liquid nitrogen-free controlled rate freezer (EF 600, Grant-Asymptote, Cambridge, UK). Finally, the MSCs for surgical administration were formulated, according to the number scheduled for each patient, after a new centrifugation at 1250 rpm for 10 min. To prepare the successive doses, cryopreserved MSCs were thawed in a thermostatic bath at 37° C, washed with antibiotic-free Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, and centrifuged at 1250 rpm for 10 min. After it, a cell count was performed and MSCs were plated at a concentration of 10,000–15,000 MSCs/cm^2 in 175 cm^2 culture flasks with antibiotic-free Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, in order to reach a confluence of 90–100% over a period of 4–5 days, and then we proceeded in the same way as with the first MSCs dose.

After obtaining the MSCs for first or successive dose administration, they were resuspended in the autologous plasma at a cell concentration of 100,000 cells/microliter. After formulation, the cell therapy medicament was packaged in sterile and endotoxins-free 1 mL-Hamilton microsyringes, with a 20G needle. Subsequently the needle is removed and a sterile luer plug nut was placed on the end of each preloaded syringes. Microsyringes with the medicament were placed inside a sterile metal box, which is also double bagged before being transported for cell administration.

2.4. Phenotypic characterization of MSCs

For phenotypic characterization of MSCs, monoclonal antibodies conjugated with different fluorochromes (Fluorescein (FITC)/Phycoerythrin (PE)/Alexa-647 (AL-647) which combine a number of both positive and negative MSCs membrane markers, were used. Positive markers used were CD105 FITC (R&D Systems,Minneapolis, USA); CD90 AL-647 (AbDSerotec,Oxford, OX5 1GE, UK); HLA Class I FITC (Cytognos, Salamanca, Spain); CD73 PE (BD Bioscience, New Jersey, USA) and CD166 PE (R&D Systems). Negative markers used were CD34 PE (BD Bioscience); HLA class IIPE (Cytognos); CD80 AL-647 (AbDSerotec); CD45 FITC (Cytognos) and CD31 FITC (Cytognos). Furthermore, suitable isotopic controls for FITC, PE (Cytognos) and AL-647 (AbDSerotec) respectively, were used as controls for specificity of the monoclonal antibodies. The labeled cells were acquired with a flow cytometer FC500 MPL Cytomics (Beckman Coulter, California, USA) using the MXP software (Beckman Coulter). Nonviable cells were discarded using the labeling reagent LIVE&DEAD (Invitrogen, California, USA), and he collected data analyzed with the CXP analysis software, version 2.1 (Beckman Coulter).

Criteria for the administration of MSCs in our patients included a viability greater than 95%, absence of microbial contamination (bacteria, fungus, virus, or mycoplasma), expression of CD105, CD90, HLA I, CD73 and CD166 for more than 90% of cells, and absence of CD34, CD80, HLA II, CD45 and CD31 (expression of each less than 5%), as assessed by flow cytometry (Fig. 1).

2.5. Statistical analysis

To study the differences between the scores of VAS, the months 1, 4, 7 and 10 were elected as time points, and the nonparametric Wilcoxon rank was used, comparing the scores of each time point with baseline. (GraphPad Prism program for Windows, v. 5.04, GraphPad Software, San Diego, CA, USA). A p-value < 0.05 was considered as significant. Bonferroni correction was applied.

3. Results

MSCs were administered to each patient according to the plan previously described. In no case do we observe any adverse events related to MSCs administration.

Table 1 shows NP scores and statistical analysis before treatment and 4, 7 and 10 months and Fig. 2 shows the evolution of these scores.

During the follow-up time, a clear reduction of the NP was recorded for each patient, except for one patient, with a baseline score of 6 that was not modified throughout the study.

The mean score of NP in the series showed an early and significant