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Research Article

Combined Olfactory Bulb Stem Cells Implant and Combined Hematopoyetics in Raquimedular Trauma

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Abstract

Considering our clinical experience in the devastating effects of the ALS (Amyotrophic lateral sclerosis) 24 patients were implanted with their own stem cells, in the spinal cord and in the cerebrospinal fluid, through a neuroendoscopic procedure. So far, there is no effective and bloodless treatment to reverse the functional sequelae of the spinal cord injury due to traumatic spinal injury. Considering the devastating effects of these injuries, we have designed a new treatment protocol for these patients who have autologous stem cells implanted in the spinal cord and CSF, immediately after being selected and purified, combined with OEC combining diferent types of precursors [1-5].

Objectives

a) To improve functional rehabilitation and quality of life in the injured spinal cord by means of the repetitive grafting of olfactory bulb cells and hematopoietic stem cells.

b) Evaluate the recovery (total or partial) of the functions commanded by the nervous system after mixed grafting.

c) Assess the morbidity associated with the procedure.

d) Protocolize pre-intra and postoperative care in the short and medium term in rachimedular injured patients.

Introduction

Neuronal death and degeneration occur prematurely in spinal cord trauma and in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), among others. The low CNS repair potential has led to the development of therapeutic strategies focused on maintaining the viability of damaged tissue, limiting the area of injury or halting the progression of a neurodegenerative disease. The term "stem cell" (CT) was coined in 1868 by Ernst Haeckel, a German biologist who used "Stammzelle" (stem cell in German) to designate the single-celled organism as the ancestor of all multicellular organisms. Today, CT, also called progenitor, mother or Stem Cells, are a group of cells characterized by their capacity for self-renewal throughout life, developing functions of cellular homeostasis and in the repair and regeneration of tissues and for responding to signals or stimuli present in the micro-environment where they are found, inducing their differentiation towards cell lines with specialized characteristics and functions [6-8]. To date, there is no ideal CT for regenerative medicine, since they present great heterogeneity [9-11]. It has been considered that the ideal CT for tissue regeneration are those obtained from internal cells of the blastocyst. However, its use in human therapy is ethically complex since it involves the destruction of donor embryos and has potential risks, since there is evidence of development of teratomas in vitro and in vivo. On the other hand, CT adults have the ability to differentiate into cells of different lineages, without developing teratomas and without ethical impediments [12-14]. Until two decades ago, it was thought that there was no neuronal replacement in adult mammals. However, today we know that neurogenesis exists from CT's located in the neurogenic niches of the subgranular zone of the hippocampus, the subventricular area of the olfactory bulb and the subependymal area of the spinal cord. Said neurogenesis is limited and depends on the activity of the astrocytes that secrete factors and cytokines, generating a microenvironment that induces neuronal development and differentiation in the hippocampus area, but that at the same time would fulfill an inhibitory role in the proliferation of Neural CT's. In the rest of the central nervous system (CNS) This dual effect translates into a restricted CNS repair response to injury and neurodegenerative diseases.

Materials and Methods

24 patients with (ALS) and 4 patients with traumatic spine injury in the last 6 months met the inclusion criteria for this study. Mean follow up of 18months. Spirometry not inferior to 60% was required. All patients were measured with an (ALS) scale. No patients with less than 17 points were included. Bone Marrow was stimulated with Filgrastim 10mg/kg Peripheral blood stem cells were collected $CD34^+$ cells were tested and selected Under general anesthesiaa spinal needle in the intervertebral space L_4-L_5, is introduced to obtain four milliliters of Cerebrospinal fluidand to suspend 2ml of Stem Cells in it, injecting in the subarachnoidal space. Then between D_8 and D_9 we introduce percutaneously a semiflexible 0.9mm neuroendoscope 1ml. of concentrated stem cells are transplanted. 2ml of concentrated stem cells are injected in the perispinal space. The remaining Stem Cells were injected through the central venous catheter. The last 4 patients were included in the OEC - stem cells combining group protocol. Our protocol was approved by the Ethical Committee of the Public National Health (MSP) of Uruguay. Patients with traumatic spinal injury withexpiratory volume and life capacity not less than 60% in spirometry. Patients aged 5 years and over, who have suffered any trauma (open or closed) with clinically proven spinal injury and image MRI, assisted in neurosurgical polyclinic of the Police Hospital will be invited to participate after signing informed consent (patient or their legal officer in the case of children under 18 years of age). The lesion may be cervical or dorsal vertebrae, 1 or 2 vertebrae, and the intervention will be performed 2 to 9 weeks after trauma to overcome the inflammatory component.

Exclusion criteria

a) Handicapped patients (dementia, morbid obesity).

b) Contamination of the graft with golden staph.

c) Untreated staphylococcus aureus nasal carriers.

d) No consent to the treatment.

Preoperative

a) Nasal exudate to rule out contamination with staphilococcus aureus.

b) The bone marrow is stimulated with Filgrastim 10mg/kg six days prior to admission, under the control of the hematologist.

c) General blood test (complete blood count with leukocytosis as a control parameter of infection, glycemia, blood coagulation, chest RX, electrocardiogram, consultation with anaesthesist).

d) The patient is admitted the night before surgery.

e) The oral route is suspended, and a peripheral venous pathway with basal input is placed.

f) Entrance to surgical block, reception by the acting team (anesthetist, ENT, neurosurgeon, pathologist anatomy, block circulators and scrubs).

Intervention

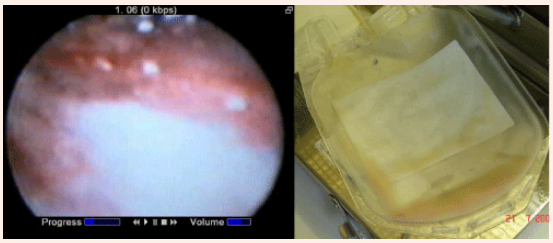
a) Surgical procedure integrates four specialties: Hemathology, pathologist, ENT and neurosurgery.

b) The Otolaryngologist will perform nasal endoscopy for the extraction of olfactory bulb cells. The pathologist anatomy will identify the OEOs.

c) Neurosurgeon by neuroendoscopy will implant the bulb cells into the topography of spinal injury.

d) The hematologist performs intravenous infusion of the remaining pool of stem cells (systemic implant)

e) The entire procedure will be performed under general anesthesia and orotracheal intubation according to the assessment of the general and anesthetic terrain (ASA score) directed by anesthesiologist.



It is emphasized that the procedure complies with the FDA's stipulations regarding the concept of Minimal Non- Cell Manipulation.

Figure 1: Stem cells harvesting and spinal graft.

f) Prophylactic antibiotic: intraoperative intravenous cefazoline 2grams (Figure 1).

Description of Endoscopic Nasal-Approach

a) Endoscopy tower is prepared and a 30th Hopkins optic is connected (Figure 2).

b) Lower and middle nasal turbinates are identified.

c) Adrenaline is infiltrated into the upper mucosal sector of the nasal septum, 1cm behind the head of the middle turbinate.

d) Dissects and prepares mucous flaps of 3-4cms in olfactory bulb area.

e) The dissected piece is sent for pathologicalstudy to certify that it is mucosa of the olfactory and bacteriological bulb to rule out contamination of it.

f) The dissection bed from which the mucosa was extracted is bloody, placing surgicell for haemostasis.

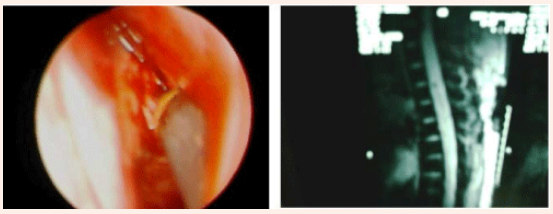


Figure 2: MRI Image of the Spinal Cord Implant.

Description of the Neurosurgical Technique

a. We close Peripheral stem cells are collected from patients through a central venous catheter We close before peripheral using a continuous flow apheresis equipment (Figures 3&4).

b. The leukocyte layer is then separated from the plasma and the $CD34^+$ cells are tested and selected. Manipulation in the laboratory of peripheral stem cells is performed immediately before the neuroendoscopic procedure in order to preserve cellular viability.

c. Once in the operating room and under general anesthesia, the patient is placed on the left side, with the dorsal spine completely bent over the edge of the surgical table.

d. The intervertebral is punctured with a spinal needle between L_4 and L_5, to obtain four milliliters of CSF.

e. The 4ml of CSF with 2ml of the pre-extracted stem cells in suspension. This suspension will be injected into subarachnoid space.

f. The intervertebral space between D_8 and D_9 is then reversed, where a percutaneous semi-flexible endoscope is introduced through a small incision by means of an introduction to recognize the posterior medial groove of the marrow, avoiding the surrounding neurovascular structures. The 1mm endoscope is inserted into the posterior middle groove and 1ml of concentrated stem cells is injected in combination with the bulb mucosa.

g. Ten minutes later, after checking that the graft is firmly attached through the formation of a clot with few red elements, 2ml of concentrated stem cells are injected into the periespinal space, ending the process of self-transplant.

h. Fibrin glue is used in the dura mater to prevent CSF fistula through the puncture needle.

i. Se closes the skin with stitches.

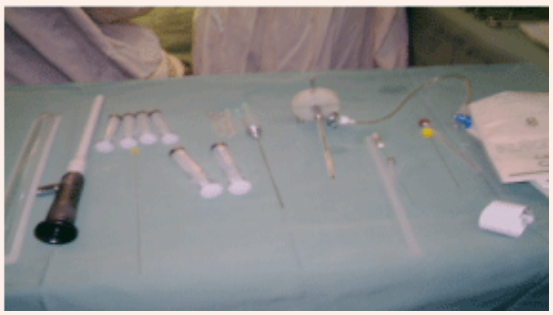


Figure 3: Neurosurgical Technique.

j. Remaining stem cells are injected through the central venous catheter, which is removed at the end of the procedure.

Postoperative

a) The patient will be driven from the surgical block to anesthetic recovery room, and then to the ward on the hospital floor.

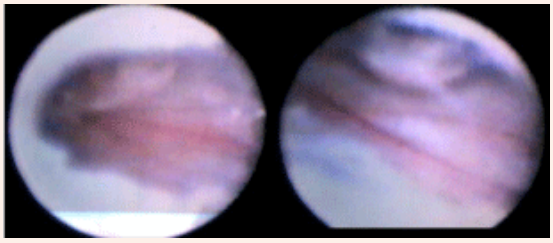


Figure 4: Endoscopic View of the Spinal Cord.

b) Once recovered from anesthesia it will test tolerance to feed and in case of good tolerance it starts with soft regimen.

c) Analgesics and antibiotics that have already started in the intraoperative period will be indicated.

d) At 24 hours, the nasal plug is removed if there was no bleeding.

e) If the developments are satisfactory, within one week high.

f) Polyclinic checks will be done a week, then monthly in the first year and then every 6 months for the next 5 years.

g) Upon discharge the patient continue with the Rehabilitation.

Results to Measure

Functional Recovery: To evaluate the recovery (total or partial) of the functions commanded by the autonomic nervous system after mixed grafting.

The evaluation is based on the ASIA and FRANKEL functional scale (see table)of motor response, per month, 3, 6, 12 and 24 months after the procedure. The occurrence of symptoms of sensory and vegetative functional neurological recovery will also be controlled: change in sensitive level, partial recovery of sphincterian function, sweating phenomena. The evaluation will be associated with somatosensitive and motor evoked potentials **(Tables 1&2)**. The control will be carried out independently by Neurologist and Physical medicine. The checks shall be carried out on the monthof the intervention 3, 6, 12 and 24months. The improvement in motor deficits will be measured on the scale of Asia. Somatosensitive and motor evoked potentials will be measured asthe most objective way to measure functional recovery and imagenological monitoring with spine MRI in each control. This has been done at the level of the different international groups that have

Table 1: Asia Scale.

Grades	Injury	Scaling
Grade A	Complete injury	Motor and sensitive commitment including S_4-S_5
Grade B	Incomplete injury	Sensitivity is preserved including S_4-S_5. There's no motor function
Grade C	Incomplete injury	Motor function preserved below the injury up to a degree of force less than 3
Grade D	Incomplete injury	Motor function preserved below the injury with a degree of force majeure of 3
Grade E	Normal	Motor and sensory function preserved

shown effectiveness in the practice will be considered that there was a complete response to treatment when it is found.

Asia scale improvement (symptom improvement in%)

i. Improvement of clinical condition.

ii. Improvement of paraclinical findings in electromyogram (EMG)).

iii. Partial response to treatment shall be considered when it is found.

iv. No change on the Asian scale.

Table 2: Frankel's Grade Forecast Classification.

Grades	Classification
Grade A	Full motor and sensory injury
Grade B	Full motor injury with some perception of sensitivity
Grade C	Motor function present, but useless for the patient
Grade D	Partial but useful motor function
Grade E	Normal motor and sensory function

v. Partial improvement of signs and symptoms.

vi. Partial changes in electromyogram (EMG).

To evaluate the morbidity associated with the procedure

The occurrence of

i. CSF fistula, manifested by rhinorraquia

ii. Fistula-associated meningitis

iii. Postoperative pain from meningeal irritation. (Probable but not reported complications in the different series and staff De Bellis et al.)

To protocol pre-intra and postoperative care in the short and medium term in rachymedular lesions who undergo graft.

List of Data to Collect

The following variables will be recorded at the time of diagnosis:

Basal Data

- Patient Name (Initials)
- Genre
- Date of birth
- Date of diagnosis of rachymedular injury
- Neurologialcurrent diagnostic
- Previous diseases
- Type of disease presentation at the beginning Initial disease presentation type

Clinical Data

- Neurológic symptoms
- Types of motor deficit:paresis-plegiascaleASIA
- Sensitive level or
- Sphincterian deficit or
- Chest pain
- Disnea

Radiological Findings

- MRI-CT

Results

No complications were observed. Periodically assessment of the evolution of the score points of the ALS function and ASIA scalerevealed at 18months that 15 patients improve their initial scores while 9 keep the same condition with no progressive deterioration. A decrease of the muscular fasciculations was observed. Three patients died with pulmonary insufficiency in the first 18months. Patients with spine injury showed improvement of motor and sensitive - vegetative performances in 3 cases, with no changes in one patient.

Discussion

The enveloping cells of the olfactory bulb (CEO), possess regenerative capacities, are part of the glía limitans of the olfactory nerve and have the ability to distribute both along the olfactory epithelium in the SNP and within the CNS, in the layers of fibers olfactory and glomerular [15-17]; they share phenotypic characteristics with astrocytes and Schwann cells [18-20]; and within the olfactory nerve, they stimulate the differentiation of neural precursors towards olfactory neurons, enabling them to reconnect with the olfactory bulb in the CNS, after injury [21,22]. CEO transplants have been used as an alternative in the repair and regeneration of CNS anisomorphic lesions (with destruction of the blood-brain barrier and the glia limitations), mainly at the level of the spinal cord, caused by: dissection, hemisection and contusion mechanics. The results obtained demonstrate neuroprotection, regeneration and partial functional recovery of the affected area [23,24]. The use of cells of the olfactory bulb to promote the repair and regeneration of damaged tissues in various neurological pathologies is proposed, for which curative therapies are currently lacking, including, among others, ischemia, Parkinson's and medullary trauma, in which both structural and functional benefits were obtained [25,26]. There is evidence of the ability to self-renew and differentiate CTs as well as their ability to secrete trophic factors, induce signals of cell survival, and interfere in the long term with the mechanisms responsible for neuronal apoptosis. Furthermore, they directly inhibit cell death by inducing anti-apoptotic and anti-oxidant proteins.

Combination of both cellular lines may promote bettercell recovery and eventual motor and sensitive sensorial functions on the basis of the homing, niches and trophic´s factors theory. Vegetative function improvementwas also observed. Surprisingly the motor and sensorial improvement was observed despite no electrical changes in evoqued potentials.

Acknowledgement

To: Prof Dr Roberto de Bellis

In Memoriam To: Mr. Sergio and Mrs. Raquel Cordoba for the support and translation.

Conclusion

The procedure was safe, feasible and easy to reproduce I with i of neurological condition was registered and stabilization of the progressive disease in others In spite of the short follow up we think is a encouraging new approach in restorative neurosurgery.

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