INTRODUCTION

Spinal cord injuries (SCI) represent a complex disorder with multiple clinical symptoms, depending on the level and complexity of the injury. In general the rehabilitation and management of SCI is a relatively recent phenomenon.\(^1\)

Cell transplantation therapies have received increasing attention in pre-clinical research, such as strategies for the treatment of spinal cord injuries. Olfactory ensheathing glia transplantation, which we reproduced in the laboratory, is a technique that has been widely studied, particularly by Ramón-Cueto, who demonstrated the ability...
of the olfactory ensheathing glia to promote functional and histological repair in central nervous system (CNS) injuries.5

Based on observations that the olfactory system exhibits neurogenesis and sends axonal processes to the CNS for life, it was suggested that the OECs possess unique regenerative properties.4 These cells enable axonal regeneration, direct the growing axons through the glial scar, have the ability to remove degenerated axons via phagocytosis, and produce channels through which recently-formed axons are guided for their regrowth, and while the OECs do not form myelin in the olfactory system, they do generate it once transplanted into the site of injury.3,5

Preclinical studies show that the effects of adult OECs are reproducible in experimental models of spinal cord lesions.6 In this study, we measured the effects of intraspinal transplantation of the olfactory bulb in a population of rats with acute spinal cord injury that can adequately simulate an acute SCI in a patient, in order to verify the usefulness of OEC transplantation in spinal cord regeneration after complete SCI, and to measure functional recovery after transplantation and physical training.7,9

Objective: To demonstrate the utility of olfactory bulb transplant in spinal cord regeneration after complete spinal cord injury. To measure functional recovery in rats with complete spinal cord section after olfactory bulb transplant. To demonstrate the axonal regeneration of complete spinal cord section after olfactory bulb transplant.

METHODOLOGY

A randomized controlled experimental prospective study was conducted, which included a sample of 15 syngeneic female adult Sprague-Dawley rats, weighing 250g. The rats were divided into thirds, which were trained for 2 weeks based on the Pavlov classical conditioning method, to strengthen the muscles of the hind feet.8 All the animals were given pre- and post-anesthetic care, except for the donor rats, which were sacrificed. One week postoperative, the training of both the case and control rats was resumed. The evolution of the experimental animals was video recorded.

Between the 80 and 120 days postoperative, depending on the clinical status of the rats, the spinal cord was removed. All the removed spinal cords were dyed with glial fibrillary acidic protein and submitted to histological immunohistochemical analysis.

It should be mentioned that the study met all the bioethical and regulatory criteria of animal protection.

RESULTS

In the immediate postsurgical period, all animals showed flaccid paralysis of both hind feet.

During the evolution, it was observed that the control rats died before 30 days of life, without showing any evidence of locomotor recovery; only 2 of the control rats managed to survive until 60 days.

Prior to its clinical deterioration and death, the spinal cord was removed for pathological study. It is worth mentioning that all the controls remained paraplegic until their deaths.

In the surgical act of removing the spinal cords, it was observed that the spinal cords of the control rats were completely sectioned, with the presence of abundant scar tissue in the skin, muscles, and subcutaneous tissue, which hampered the surgical approach. (Figure 1)

All the rats case evolved in a similar manner in terms of locomotor recovery: claw flexion of the feet at around day 12 postoperative, normal position on 4 feet by day 30, mobility of the thighs and ankles by day 60, and return to walking on the hind feet at around day 90 postoperative, with weak and unstable gait, but at 140 days, the hind feet regained strength and the angle of mobility increased. (Table 1, Figure 2)

Table 1. Evolution postoperative.

<table>
<thead>
<tr>
<th>Rat (Case)</th>
<th>12-14 days</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Claw flexion of the toes (bilateral).</td>
<td>Flexion of the thigh and ankle, standing on 4 feet (original position). No strength.</td>
<td>Movement of the thigh, ankle and hip, walking on 4 feet. Recovery of strength.</td>
<td>Died at 80 days. Extraction of the spinal cord before death.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Claw flexion of the toes (bilateral).</td>
<td>Flexion of the thigh and ankle, standing on 4 feet (original position). No strength.</td>
<td>Movement of the thigh, ankle and hip, walking on 4 feet. Recovery of strength.</td>
<td>Walks on 4 feet, speed and coordination of the movements of the hind feet recovered.</td>
<td>Drags the body with the hind feet (coordinated and rapid flexion and extension of the thigh, knee and the hip).</td>
</tr>
<tr>
<td>3</td>
<td>Bilateral claw flexion of the right muscle.</td>
<td>Bilateral flexion of the thigh and ankle, standing on 4 feet (original position). No strength.</td>
<td>Bilateral movement of the thigh, ankle and hip, walking on 4 feet. Recovery of strength.</td>
<td>Walks on 4 feet. Recovery of speed and coordination of the movements of the hind feet.</td>
<td>Drags the body with the hind feet (coordinated and rapid flexion and extension of the thighs, knees, and the hip).</td>
</tr>
<tr>
<td>4</td>
<td>Bilateral claw flexion of the toes.</td>
<td>Flexion of the left thigh and ankle, standing on 3 feet, the right foot remains extended behind.</td>
<td>Movement of the left thigh, ankle and hip (flexion and extension), walking on 3 feet. Recovery of strength.</td>
<td>Bilateral movement of the thigh, ankle and hip (flexion and extension), walking on 4 feet. Recovery of strength.</td>
<td>Walks on 3 feet with greater speed and coordinated movements. Increased strength of the right hind foot.</td>
</tr>
<tr>
<td>5</td>
<td>Bilateral claw flexion of the toes.</td>
<td>Bilateral flexion of the thigh and ankle, standing on 4 feet (original position). No strength.</td>
<td>Bilateral movement of the thigh, ankle and hip (flexion and extension), walking on 4 feet.</td>
<td>Bilateral movement of the thigh, ankle and hip (flexion and extension), walking on 4 feet. Recovery of strength.</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. A) Control rat first week postoperative, complete paraplegia of the hind feet is observed. B) Control rat 30 days postoperative, complete paraplegia of the hind feet continues. C) 60 days postoperative, there are no signs of locomotor recovery.
During the surgical procedure to remove the spinal cord, the scar tissue was minimal, facilitating the procedure and enabling the spinal cord to be more easily dissected. Once the spinal cord was exposed, its continuity through the transplantation site was observed, without identifying the olfactory bulb or the gap between the extremities of the sectioned area, resembling an intact spinal cord.

Histological studies showed the presence of neural tissue at the site of the lesion, with few glioses in the case samples, which were stained with hematoxylin and eosin.

Immunohistochemically, in the samples dyed with glial fibrillary acidic protein, evidence of continuity of the axonal tissue was observed, indicating regeneration of the spinal cord in the case samples. (Figures 3 and 4)

No control sample showed findings of axonal regeneration, but abundant glioses were found.

CONCLUSION
Intraspinal transplantation of the olfactory ensheathing cells improves locomotor function and stimulates axonal regeneration in rats with acute spinal cord injuries, suggesting its possible efficacy in humans. However, spinal cord injury in laboratory animals cannot accurately model the inherent heterogeneity of the neurological damage seen in patients, nor is it possible to objectively assess all aspects of spinal cord function.

These findings prompt us to continue to conduct research on this subject, as they are important for the development of future treatments for spinal cord injured patients, which so far remains an incapacitating condition for life.

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