AN EXPERIMENTAL MODEL FOR THE TRANSPLANTATION OF FETAL CENTRAL NERVOUS SYSTEM CELLS TO THE INJURED SPINAL CORD IN RATS

Tarcísio Eloy Pessoa de Barros Filho, Reginaldo Perilo de Oliveira, Ana Maria Tsanaclis, Erika Meirelles Kalil Pessoa de Barros, Alexandre Fogaça Cristante, Ricardo Moreira Palma, Cristian Vilela dos Santos and Raphael Martus Marcon

BARROS FILHO TEP de e col. - An experimental model for the transplantation of fetal central nervous system cells to the injured spinal cord in rats. **Rev. Hosp. Clín. Fac. Med. S. Paulo 57**(6):257-264, 2002.

INTRODUCTION: Traumatic spinal cord injury is one of the most disabling conditions occurring in man and thus stimulates a strong interest in its histopathological, biochemical, and functional changes, primarily as we search for preventive and therapeutic methods.

PURPOSE: To develop an experimental model for transplantation of cells from the fetal rat central nervous system to the site of an injured spinal cord of an adult rat in which the transplanted cells survive and become integrated. This experimental model will facilitate investigations of factors that promote regeneration and functional recovery after spinal cord trauma.

MATERIAL AND METHODS: Fifteen adult Wistar rats underwent laminectomy, and an spinal cord lesion was made with microdissection. Fetal spinal cord tissue was then transplanted to the site of the injury. The rats were monitored over a 48-hour period, and then their vertebral column was completely removed for histological analysis.

RESULTS: In 60% of transplanted rats, the fetal tissue at the injured site remained viable in the site of the lesion.

DESCRIPTORS: **Spinal cord injury. Fetal cells. Rats.**

INTRODUCTION

Spinal cord injury has been considered a condition without the possibility of successful treatment[15]. However, emerging studies in this field indicate that acute spinal cord injuries can be minimized by the use of pharmacologic therapy when drugs are administered within a short period of time following the trauma[9,23,26,60]. This progress is attributable largely to histological observations, which have facilitated better understanding of the sequence of events involved in the spinal cord injury[10].

The first experiments focusing the pathophysiologic mechanism of the injured spinal cord were performed at the beginning of this century[1, 2]. Nevertheless, these works were resumed only in the past decade by investigators who have begun to value the time-dependent changes implicated in the physiopathology of spinal cord trauma[3,6,48].

The significant neurological deficit related to the spinal cord injury is caused by the sum of two different conditions: the initial mechanical injury and the secondary endogenous injury resulting from the initial injury[6,7,20,22,32,47,59,63]. The primary damage is caused by the trauma itself and involves either cellular death or electrolyte, metabolite, and enzyme release. It is therefore a mechanical process that does not depend on cellular control mechanisms. The secondary injury of the spinal cord involves complex biochemical changes, which occur as a cascade of events such as swelling, inflammation, ischemia, reperfusion, the presence of growth factors, and calcium and lipidic peroxidase metabolism, and on which scientific ef-

From the Department of Orthopaedics and Traumatology, Hospital das Clínicas, Faculty of Medicine, University of São Paulo.

forts are based to reduce secondary injury[7,8,24,25,34,42,43,46,53,57,58,61,63]. From a pharmacological standpoint, drugs that modulate the endogenous responses to the primary injury have been progressively introduced into therapeutic protocols to restrict the collateral damage and improve the potential for functional recovery of these patients. These drugs are administered to interrupt the pathophysiologic mechanisms of the secondary neuronal injury[27,28,51].

Clinical and scientific advancements indicate that acutely injured spinal cords can be managed by pharmacological therapeutic procedures used within a short period of time. Methylprednisolone administered within the initial 8 hours after the trauma is the first pharmacological agent to promote significant improvement in the recovery of the injured spinal cord in human beings[4,11-14,19,35-40,52,56]. Other drugs, such as tirilizad[5,39,41] and GM-1[18, 30, 31, 33, 49, 50, 64], which are still under clinical investigation, demonstrate promising preliminary results. These advancements should represent a significant improvement in the quality of life of patients with spinal cord injury once they are employed in current medical practice.

Central nervous system injuries are followed by a deficit condition and a period of variable functional recovery. Such recovery is strongly related to changes that involve unharmed circuits, although the exact mechanism of the recovery has not been completely clarified. Transplanting neural cells is helping investigators to understand the development of the CNS and its response to injuries. More recently, such transplants have been used to optimize the posttraumatic functional recovery. The specific mechanism through which such transplants act is not completely understood. Present theories posit the influence of trophic activity along with the release of hor-

mones and neurotransmitters, and the re-enervation of host cells by the transplanted cells[16]. Current research is intended to determine the degree of recovery that can be achieved by these transplants.

The possibility of employing fetal nervous system cells for the treatment of a number of CNS pathologies has stimulated several studies on the physiology of the survival and integration of the transplant. Today it is known that transplanting fetal cells potentiates the locomotor recovery both in immature and adult individuals; However, the mechanism responsible for this observation is still unknown[44].

Several protocols for transplantation of fetal nervous system cells have been reported, but there is not a consensus among physicians on the best method [44].

OBJECTIVE

The purpose of this study was to develop a reproducible method for transplanting cells from the fetal rat central nervous system to the site of an injured spinal cord of an adult rat that results in the survival and integration of the transplanted cells. Using this methodology, researchers will be able to study other factors that support regeneration and functional recovery of the posttraumatic spinal cord.

MATERIALS AND METHODS

Model of an Injured Spinal Cord and Donor Tissue Preparation

One male and two female rats were selected and put into 5 cages. After 12 hours, vaginal swabs from the female rats were taken and analyzed under optical microscopy to verify the presence of spermatozoids. The female rats

whose vaginal swabs presented evidence of spermatozoids were considered pregnant and isolated in a different cage.

The donor tissue was obtained from fetal rats obtained by cesarean section on Day 14 of gestation. Immediately after the cesarean, each fetus had its central nervous system removed, which was inoculated into the injured spinal cord of the adult rats.

We analyzed the injuries from 15 rats initially, which were produced through hemilaminectomy at the T10 level and aspiration and delicate 05-mm segment microdissection from the rat spinal cord.

The donor tissue was transplanted into the site of the injury using micropipettes, and it was then properly sutured.

The rats were sacrificed 48 hours following transplantation, and their spinal cords were surgically excised and submitted to histopathological analysis to verify the viability of the transplanted cells.

Rats

We used male and female Wistar rats aged 20 weeks and weighing 270 to 315g and 200 to 280g respectively, which were from a single supplier. They were acquired 1 week prior to the surgery to become acclimatized and manageable.

Laminectomy

The spinal cord was exposed through a laminectomy as follows:
- An opening was made in the median dorsal line of the skin to expose the vertebral column at T10 level (Fig. 1A) .
- The muscles inserted in the spinous processes from T9 to T11 level were sectioned and separated using a bipolar coagulant device to stop any bleeding if necessary.

The T10 vertebra and the distal half of the T9 spinous process were re-

moved using a small punch. The removal began by the caudal edge of T10. Small fragments were delicately removed using the punch guided from the cranial to the caudal half of the sheath at the T9 level. (Fig. 1B). The spinal cord was not harmed (Fig. 2) .

The spinal cord injury procedure
• A 0.05 mm segment of one half of the medula of the rat was removed by aspiration and microdissection using a microscope.
• The rat was removed and placed on

a warm surface. The contusion site was inspected. Any bleeding was stopped, and the contusion site was washed with a saline solution.

Material for the transplantation
The female rats underwent a cesarean section (Fig. 3A) . The fetuses were removed, and embryonic cells of the CNS were harvested with a microsurgical technique (Fig. 3B). The material that was obtained was obliquely sectioned into 0.05-mm segments for transplanting into the injured site.

Transplantation of fetal cells into the injured site
• The segment of the fetal CNS that was previously sectioned was implanted into the site of the injured spinal cord of adult rats.
• After the transplantation, the dural sac was closed using fibrin glue. Muscular, subcutaneous and cutaneous tissues were sutured.

Posttraumatic procedures
Over a 48-hour period following the injury, the rats were observed and had their deficits registered.

Euthanasia procedure and taking tissue samples for acute experiments
Euthanasia was performed 48 hours after surgery. The procedures for the euthanasia and the removal of tissue samples were as follows:
• 48 hours after the injury and transplantation, the rat body weight was registered at the time of sacrifice.
• The rat was anesthetized using pentobarbital 40 mg/kg given intravenously.
• The aorta of the rat was catheterized through thoracotomy to allow perfusion with paraformaldehyde solution.
• The vertebral column from C5 to L5 level and most of the muscles were quickly removed (Fig. 4).
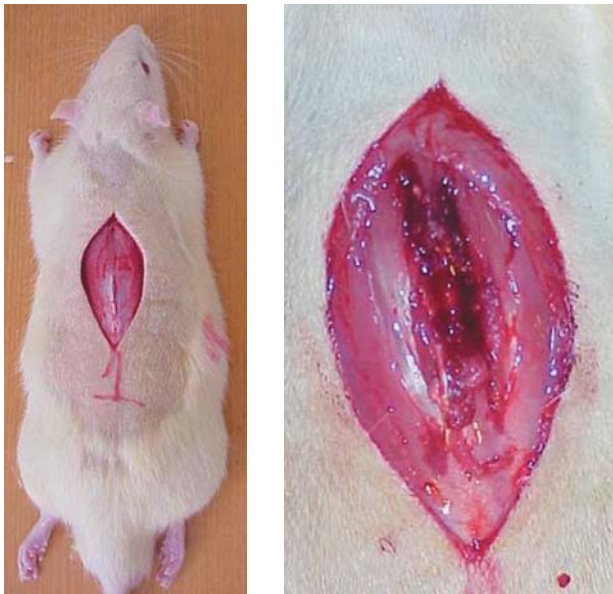
Figure 1 - A – Approach via to the vertebral column. B – Laminectomy.

Figure 2 - Spinal cord exposure.

Figure 3 - A - Exposure of the pregnant uterus through cesarean section. B - Fetus.

• The vertebral column was placed in a centrifuge, covered, and taped with *Parafilm®*.

Figure 4 – Removal of the vertebral column from the C5 to L5 level.

Necropsy

After sacrifice, which occurred 48 hours after the spinal cord was damaged and fetal cell transplantation was performed, the animals were weighed and the identity and gender were confirmed. Then, the vertebral column and the spinal cord were removed.

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Histological analysis of the transplanted spinal cord

Histological sections were obtained from the area of the spinal cord containing the transplanted cells. The magnitude of the injury and/or the presence, placement, and viability of the implanted cells were analyzed.

RESULTS

The breeding procedure resulted in 4 female rats with vaginal swabs positive for spermatozoids. They all became pregnant, and on day 14 of gestation, the rats underwent a cesarean section and were sacrificed.

After the production of the spinal cord injury, all the rats presented evidence of neurological deficits, which varied from complete monoplegia to complete paraplegia. The clinical finding of the magnitude of the spinal cord injury was later correlated with its histological finding.

All the rats were still alive 48 hours after the fetal cells transplantation. Also, there was no evidence of autophagia, pressure ulcers, or infection.

The histological analysis of the spinal cord sections relating to the site of the injury showed that the spinal cord damage resulting from the microdissection was not constant, varying from 40% to subtotal injury (Fig. 5). In 3 cases, a hematoma was found at the injured site, which was caused by a broken blood vessel (in this case, the anterior spinal artery).

The analysis of the histological sections showed the presence of the implanted fetal cells after a 48-hour period. However, in 40% of the cases, fetal cells were not identified at the site of the injury, but only in its trajectory (Table 1 and Fig. 6).

DISCUSSION

Traumatic spinal cord injury is one of the most disabling conditions occurring to man, which stimulates a strong interest in its histopathological, biochemical, and functional changes, mainly when we are searching for pre-
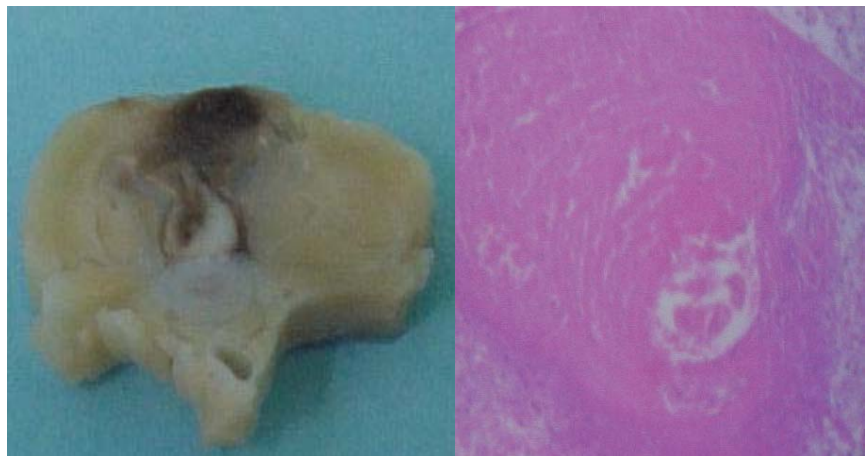
Figure 5 - A – Macroscopic section of the vertebral column showing the lesion and a hematoma in the trajectory of the lesion. B – Microscopic section showing the hematoma.

Table 1 - Viability and place of the fetal cells transplantation.

RAT	TRANSPLANTED FETAL CELLS VIABLE	TRANSPLANTED CELLS	AT THE INJURY SITE AT THE INJURY TRAJECTORY
1	Yes	Yes	Yes
2	Yes	No	Yes
3	Yes	Yes	Yes
4	Yes	Yes	Yes
5	Yes	No	Yes
6	Yes	No	Yes
7	Yes	Yes	Yes
8	Yes	No	Yes
9	Yes	No	Yes
10	Yes	Yes	Yes
11	Yes	Yes	Yes
12	Yes	No	Yes
13	Yes	Yes	Yes
14	Yes	Yes	Yes
15	Yes	Yes	Yes
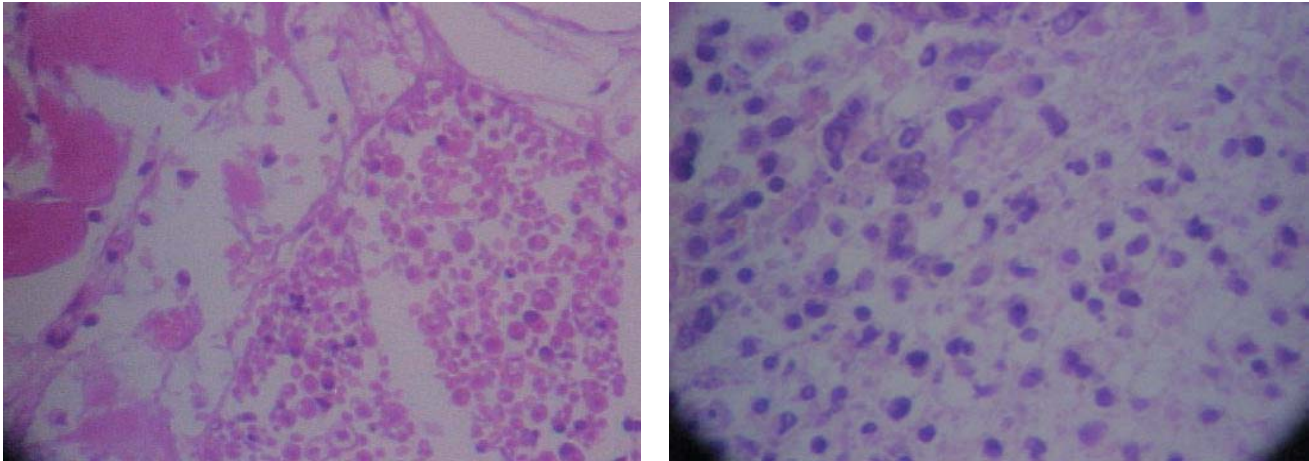
Figure 6 - A – A Microscopic section showing groups of fetal cells at the site of the injury. B – A smaller enlarged image of a section from a group of fetal cells at the site of the injury.

ventive and therapeutic methods for managing the sequelae of the spinal cord trauma.

For treating the spinal cord injury in man, experimental models are necessary for testing drugs, surgery techniques, and other therapeutic procedures, such as cell transplantation to the injured site. In the literature, there is not a universally accepted model of experimental spinal cord injury, which is attributable largely to different parameters that are analyzed and the significant diversity of therapeutic techniques that are tested.

Open experimental spinal cord injuries produce the best conditions for study. Closed injuries produce fractures whose fragments change the natural course that is intended for study. The spinal cord that is exposed can be directly sectioned or contused. Experimental methods that use different impacts as causative agents for the spinal cord injury include falling weights, crushing of the spinal cord using special tweezers for aneurysm[57], extradural balloons that are gradually inflated, and damage caused by radiofrequency and microdissection. The ideal model would include the traumatic mechanism usually found in human beings, reproducibility, and the possibility of a quantitative analysis.

Nevertheless, such model has not yet been described.

We used a laminectomy technique and the 05-mm microdissection of one half of the medula in this preliminary study, and we analyzed the placement and the viability of a 05-mm segment of fetal CNS implanted into the site of the injury.

Although the microdissection was performed using a microscope, the histological analysis of the spinal cord injury showed evidence of differences in the magnitude of the injuries that were produced, which were usually larger than 50% of the spinal cord. These differences did not impair the objective of this study, which was to evaluate the histological presence of fetal cells 48 hours after the transplantation. However, for better standardization of the injury, subsequent studies will have to focus on the chronic phase of the injured spinal cord using functional evaluations through locomotion scales, electrophysiological, and pathological assessments.

We chose using Wistar rats because of their good availability and the minor technical difficulties in handling these animals. The preferred species for experiments involving spinal cord injury is one of the nonhuman primates, but their use is restricted due to

high costs, little availability, handling difficulties, and ethical considerations[7]. Rats are a good alternative in these experiments because their spinal cord has a cytoarchitectural organization and vascularization similar to that of humans[7].

Transplantation of cells from the central nervous tissue has been used for the past 20 years and has helped to increase understanding concerning the development of the nervous system and the neuronal response to injury. More recently, studies involving transplantation of cells from the central nervous system have been performed to restore or reduce the loss of function resulting from the injury. It has been proven that transplantation can reduce deficits or even increase the functional recovery following damage of the central nervous system, mainly in cases of degenerative diseases. Transplantation can influence the recovery of the function after the CNS trauma through several mechanisms including non-specific consequences of transplantation, trophic actions, hormone and neurotransmitter release, and more specific mechanisms involving re-enervation of host cells and establishment of reciprocal connections between the transplanted and the host tissue[16,17].

The requirements for anatomical and functional recovery after spinal cord injury are more complex than from other neurological damage, which usually requires only restoration of the neurotransmitter levels [16].

There are several mechanisms through which the transplantation of fetal cells can affect the response to the injury and mediate the functional recovery after the injury. The transplanted fetal cells from the central nervous system are able to connect the spinal cord with the supraspinal structures through the site of the injury. The transplanted cells act as a substratum for restoring cellular communication between upper and lower levels of the injured tissues. At the cellular level, transplantation can supply trophic support either for mature or immature neurons, inhibit the formation of a glial scar at the site of the injury, and supply a favorable mechanical substratum of extracellular matrix for neuronal growth[16].

Transplantation using cells from the central nervous system can improve the locomotor function after a spinal cord injury and provide a more complex microenvironment than that offered by peripheral nerve transplantation, cell suspensions, or genetically altered cells[44].

In our study, the histological analysis of the injured site showed the presence of viable fetal cells in 9 of 15 rats (60% of cases) that underwent the transplantation of fetal cells from CNS. In 40% of the cases fetal cells were not found at the site of the injury, but only in its trajectory.

This study showed the potential of a rat model of using transplanted fetal spinal cord cells that remain viable for 48 hours after their transplantation. Additional studies on the chronic phase of the spinal cord injury and the short- and long-term viability of fetal cells using functional assessments and histopathological evaluations are planned.

RESUMO

RHCFAP/3103

BARROS FILHO TEP de et al. - Modelo experimental de transplante de células do sistema nervoso central fetal para lesão de medula espinal em ratos. **Rev. Hosp. Clín. Fac. Med. S. Paulo 57**(6):257-264, 2002.

INTRODUÇÃO: A lesão traumática da medula espinal consiste numa das mais incapacitantes lesões que o ser humano pode sofrer e tem despertado grande interesse no conhecimento das alterações histopatológicas, bioquímicas, funcionais e principalmente na busca de métodos de prevenção e tratamento.

OBJETIVO: Propor um modelo experimental de transplante de células do sistema nervoso fetal de ratos para o sítio de lesão medular de ratos adultos que permitisse sua sobrevivência e integração para possibilitar protocolos de pesquisa para identificar outros fatores de regeneração e recuperação funcional pós trauma raquimedular.

MATERIAL E MÉTODOS: Utilizaram-se 15 ratos adultos que foram submetidos a laminectomia e lesão de 5mm de hemimelula realizada com auxílio de microscópio óptico. Os ratos tiveram seu sítio de lesão medular transplantado com células do sistema nervoso central de fetos de rato. Os ratos foram monitorados por 48 horas e tiveram sua coluna vertebral extraída para análise histológica.

RESULTADOS: Demostrou-se que em 60% dos casos as células transplantadas permaneciam viáveis no sítio da lesão.

DESCRITORES: **Lesão medular. Células fetais. Ratos.**

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