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Subarachnoid Placement of Stem Cells in Neurological Disorders

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ABSTRACT

Background. "Medically untreatable neurological disorders" is an area where stem cell (SC) therapy has generated hope in the last decade. Among various routes for SC infusion, subarachnoid placement via the lumbar route is particularly challenging because of technical difficulties in this group of patients. We carried out a prospective, single-center, clinical study to analyze the technical difficulties and short- and long-term effects of SC infusion in various neurological conditions.

Patients and Methods. One hundred eighty patients underwent subarachnoid placement of SCs between December 2005 and October 2007. Technical difficulties in the form of localization of subarachnoid space, number of attempts, and postprocedural complications were evaluated. Functional evaluation was done with Hauser Ambulation Index by the SC transplant team on a regular basis. The Institutional Review Board approved of informed consent forms and study protocol.

Results. Of 180 patients, we encountered technical difficulties in 52 (29%) in the form of general anesthesia supplementation and difficulty localizing the lumbar space. In 102 (56.6%) patients, side effects were observed (headache, low-grade fever, and meningism), which resolved with symptomatic treatment within 24 hours. On long-term follow-up, functional indices improved in 57 (31.67%) patients, including 54 patients with traumatic paraplegia/quadriplegia, two with cerebral palsy, and one with viral encephalitis.

Conclusion. Subarachnoid placement of SCs is safe with no long term adverse effects.

DURING THE LAST DECADE, stem cell (SC) biology has branched into new areas where there are no definitive medical therapeutic strategies. Neurological disorders along with congenital anomalies are areas where SC therapy has generated hope. The reparative, regenerative, and replicative properties of SCs have shown the potential for treatment in various neurological conditions. We have used SC therapy for many medically untreatable neurological conditions, such as posttraumatic paraplegia/quadriplegia, cerebral palsy, motor neuron disease, and multiple sclerosis (unpublished data). Among various routes for SC infusion, subarachnoid placement via the lumbar route is particularly challenging because of technical difficulties in this group of patients. We carried out a prospective analysis to assess the technical difficulties and short- and long-term effects of SC infusion.

METHODS

After Institutional Review Board approval and informed consent, 180 patients with various neurological disorders of different duration were enrolled in the study. The source of SCs was either autologous or from a near relative of the patient within the same blood group. SCs used were adipose tissue-derived mesenchymal cells (h-AD-MSCs), human embryonic stem cell-derived hematopoietic stem cells (HESC-HSCs), and autologous bone marrow-derived hematopoietic stem cells (BM-HSCs). Patients with local/systemic infection were excluded.

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Patients' X-rays, computed tomography scans, and magnetic resonance images of spine; were reviewed prior to the procedure, and the space closest to the injury site was chosen for SC placement. The subarachnoid space was localized in lateral/sitting position with 22-guage Quincke's spinal needle under all aseptic precautions and monitoring. After the free flow of cerebrospinal fluid (CSF), 7 to 10 mL of SC inoculum was infused after diluting with CSF by barbotage method. The needle was left in the same position for 2 minutes before withdrawal. Pediatric and uncooperative patients were given general anesthesia before performing lumbar puncture. The anesthesia technique used was a combination of intravenous sedation and inhalation. Midazolam and Fentanyl were used for sedation, while O_2, N_2O, and Sevoflurane were used for maintenance. Technical difficulties in the form of localization of subarachnoid space, number of attempts, general anaesthesia supplementation, and postprocedural complications were evaluated and recorded. All patients were monitored in the critical care unit for 24 hours and hydrated with 3 L of fluid; ambulation was allowed 48 hours postprocedure. Short- and long-term functional evaluation was done with Hauser Ambulation Index (HAI) by the SC transplant team on a regular basis.[1]

RESULTS

Of 180 patients, 163 had spinal cord injury, six had cerebral palsy, four had motor neuron disease, and five, other encephalopathies. Ninety-nine patients received HESC-HSCs and BM-HSCs and 81, h-AD-MSCs and BM-HSCs. Of 163 spinal cord injury patients, 46 had previous history of spinal surgery. Four required C-arm guidance, two needed Taylor's approach, and 40 required >1 attempt for localization of subarachnoid space. All six children with cerebral palsy were administered SCs under short general anesthesia. Ninety-six patients had postprocedural headache, which was relieved within 24 hours with analgesics, hydration, and rest; four had low-grade fever lasting for <24 hours; and two patients had signs of meningism, which responded to within 4 hours symptomatic treatment. On long-term follow-up, functional indices improved in 57 (31.67%) patients, including 54 of patients with traumatic paraplegia/quadriplegia, two with cerebral palsy, and one with viral encephalitis. Cerebral palsy and viral encephalitis patients showed improvement in muscle tone, rigidity, and spasm (Table 1). In traumatic paraplegia/quadriplegia patients, 42 (25.77%) patients had an injury period of <4 years, whereas 12 (7.36%) had injury period of >4 years. Thirty-two patients had improvement in motor power (24 with injury period of <4 years and 8, >4 years). Before treatment, all these patients were HAI grade 9. After SC transplantation, one was grade 4; three, grade 5; three, grade 6; and 17, grade 7. Dramatic improvement was seen in four bed-ridden patients who were able to walk with the help of a walker (HAI grade 9 → HAI grade 4/5). In 22 patients, autonomic improvement was seen, including 18 patients who had <4-year injury period. Of these, six became catheter-free and 12 required intermittent catheterization. All 22 patients had improvement in bowel sensations and sweating. A mixed motor and autonomic improvement was seen in 10 of 54 patients (Table 2).

Table 1. Results

Number of patients	180
Paraplegia	156
Quadriplegia	7
Cerebral palsy	6
Motor neuron disease	4
Miscellaneous	7
Number of patients with technical difficulties	52 (29%)
GA supplementation	6
C-arm guided	4
Taylor's approach	2
Multiple attempts	40
Side effects	102 (56.6%)
Headache	96
Low-grade fever	4
Meningism	2
Improvement in functional indices	57 (31.67%)
Paraplegia/quadriplegia	54
Cerebral palsy	2
Viral encephalitis	1

DISCUSSION

There is a valid experimental basis for SC therapy for patients with central nervous system (CNS) injuries.[2,3] The two basic approaches by which injured CNS is repaired by SC: conditions are made favorable favorable for nerve fiber

Table 2. Traumatic paraplegia/quadriplegia profile

Number of patients	163
MRI diagnosis	154
Pattern of injury	
Complete transection	20%
Nontransection	80%
Injury to treatment duration	4 mo–14 y
Clinical and functional benefit	
<4 y	42 (25.77%)
>4 y	12 (7.36%)
Pattern of motor improvement according to HAI*	
<4 y (n = 24)	
Posttransplant grade	
4	1
5	3
6	3
7	17
>4 y (n = 8)	
Posttransplant grade	
6	1
7	2
8	5
Autonomic improvement (n = 22)	
<4 y (n = 18)	
Catheter free	6
Intermittent catheterization	12
Bowel sensations and sweating	16
>4 y (n = 4)	
Intermittent catheterization	4
Improved bladder tone	4

MRI, magnetic resonance imaging; HAI, Hauser Ambulation Index.
*Thirty-two patients were HAI grade 9 before transplantation.

growth and destroyed neurons are replaced by new, functionally active neural cells. There is accumulating evidence that intervention with SCs can enhance the factors favoring axonal growth from recipient neurons and generate functionally active donor neurons. This reparative potential of grafted SCs may greatly improve outcome of the disease. An effective CNS repair appears to require at the injured site the presence of not only neural cells potentially able to provide axonal growth but also other cells capable of creating the microenvironment favorable for both growth and myelination of nerve fibers.[2,3] Undifferentiated SCs excrete a variety of neurotropic factors that encourage axon growth, promote the breakdown of glial scar, replace damaged nonneural structures such as blood vessels, and temper the inflammatory respons. Embryonic SCs in particular have a penchant for adopting the glial phenotype; they will readily transform into the support cells required by the neurons (eg, astrocytes, oligodendrocytes) once they are transfused into the site of injury.[2]

Migration toward pathology is the first critical step in SC engagement during regeneration, and it is hypothesized that the inflammatory response itself guides the behavior of potentially reparative SCs. It has been found that introducing SCs into the subarachnoid space of the spinal cord will transport the cells through CSF and allow more efficient delivery of cells to the injured area of CNS compared to the intravenous route. Intrathecal SC administration is safe, less invasive, and a convenient procedure involving no surgery. However, the efficacy of SC transplantation into the subarachnoid space depends on a grafting method that will optimize the survival of transplanted cells. The mechanical process of grafting into the CSF itself can cause damage that could diminish the viability of the transplanted cells. So it is essential to ensure excellent viability of SCs prior to transplantation. We assessed the viability with the trypan blue exclusion test. For transplantation of cells into the CSF, the patient should ideally be positioned so as to make intervertebral spaces palpable for lumbar puncture. It is essential to prevent clogging of the needle when cells settle. It is generally agreed that the best way to inject cells is to slowly deliver a fixed volume at a constant rate. Cell density and total number of cells transplanted need to be established to ensure an adequate number of cells for grafting and optimal survival. After the cells have been injected, the needle tip should be left in the same position for at least 2 minutes before withdrawing slowly to prevent any backflow.[3]

Although SC infusion into the subarachnoid space is a relatively simple procedure, an experienced anesthesiologist is required to perform this procedure due to various problems encountered in these patients. In our study, a significant number of patients had traumatic paraplegia; performing a lumbar puncture in these patients was technically difficult because of various reasons, such as previous spine surgery leading to fibrosis and adhesions, presence of plate, narrow/fused intervertebral spaces, pathological scoliosis, positional difficulties, and so on. Cerebral palsy patients need special attention because of multisystemic involvement making them ASA risk III patients. Cognitive, communication, and behavioral problems along with coexisting diseases and drug therapy influenced the anesthetic management of these patients as they had to be given general anaesthesia. Gastroesophageal reflux, salivary drooling, electrolyte imbalance, difficult airway with risk of pulmonary aspiration, and spasticity all added to our problems. Inadequate anesthesia and analgesia could further lead to increased muscle tone and spasm, making positioning the patient difficult. Therefore, adequate preoperative preparation and judicious use of an anesthetic agent intraoperatively was critical to ensure a relaxed perioperative and postoperative period.[4] A significant number of our patients ($n = 96$) had postprocedural headache. This was probably due to the use of a large-bore spinal needle (22 gauge), which was essential to prevent any mechanical damage to the cells during infusion. Two patients had signs of meningism, probably due to meningeal irritation; however, they recovered early. All the side effects resolved within 24 hours with no long-term sequelac.

The limitation of our study was that the study group was very heterogeneous in clinical presentation, duration of injury (4 months to 14 years), and follow-up time period, which was a major constraint in forming a clinically comparable control group. Review of other research studies have suggested multiple SC infusions, whereas we used a single infusion, which could have been another limiting factor. Despite of these limitations, our results are encouraging as 31.67% of patients showed neurological improvement. Further trials with clinically comparable groups are required to recommend its use in neurological disorders.

In conclusion, this study suggests that subarachnoid placement of SCs in neurological disorders has a major potential as it is a relatively safe and simple procedure with no long-term adverse effects. However, given the paucity of clinical studies that exist, this therapy has not yet realized its full potential. Nevertheless, the encouraging results provide compelling evidence to support the concept that in patients with profound neurological defects and inefficient conventional cure, there is a promise of restoration of lost tissue and improvement of function.

REFERENCES

1. Hauser SL, Dawson DM, Lehrich JR, et al: Intensive immunosuppression in progressive multiple sclerosis. A randomized, three arm study of high-dose intravenous cyclophosphamide, plasma exchange, and ACTH. N Engl J Med 308:173, 1983

2. Stem cells for spinal cord injuries. Available at: http://scienceblogs.com/neurotopia/2006/07/stem_cells_for_spinal_cord_inj.php

3. Kulbatski I, Mothe AJ, Nomura H, et al: Endogenous and exogenous CNS derived stem/ progenitor cell approaches for neurotrauma. Curr Drug Targets 6:111, 2005

4. Nolan J, Chalkiadis GA, Nomura H, Low J, et al: Anesthesia and pain management in cerebral palsy. Anesthesia 55:32, 2000