Umbilical cord mesenchymal stem cells in neurological disorders:
A clinical study

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We investigated the intrathecally administrated unbilical cord mesenchymal stem cells (UC-MSCs) by lumbar puncture and assessed the technical difficulties and effects in various neurological conditions. One hundred patients underwent subarachnoid placement of UC-MSCs between December 2006 and May 2010 in the Affiliated Hospital of Medicine. Technical difficulties in patients in the form of localization of subarachnoid space, number of attempts, and post-procedural complications were evaluated. Functional evaluation was done using Hauser Ambulation Index (HAI) by the stem cell transplant team on a regular basis. All patients were followed-up for more than 1 yr after the treatment. Clinical symptoms, related biochemical index and photographic examinations were observed regularly. We encountered technical difficulties in 31 patients (31%) in the form of general anesthesia supplementation and difficulty localizing the lumbar space. Side effects (headache, low-grade fever, low back pain and lower limb pain) were observed in 22 (22%) patients, which were treated with symptomatic therapy within 48 h. One year after the treatment, functional indices improved in 47 patients (47%): 12 patients with spinal cord injury, 11 patients with cerebral palsy, 9 patients with post-traumatic brain syndrome, 9 patients with post-brain infarction syndrome, 3 patients with spinocerebellar ataxias, and 3 patients with motor neuron disease. In conclusion, intrathecal administration of UC-MSCs is a safe and effective way to treat neurological disorders. Our encouraging results of intrathecal administration of UC-MSCs indicate the potential of restoration of lost tissue and improvement of function in patients with profound neurological defects and inefficient conventional cure. These data support expanded double-blind, placebo-controlled studies for this treatment modality.

Keywords: Neurological diseases, Umbilical cord mesenchymal stem cells, Stem cell, Intrathecal administration, Lumbar puncture.

Major human brain and spinal cord injury remain serious problems that currently have no effective treatment. Stem cells have the potential to induce neurorestorative processes, including neurogenesis, angiogenesis and synaptic plasticity that are essential for facilitating recovery of neurological function. The advances in stem cell biology have generated intense interest in the prospect of transplanting stem cells into the nervous system for the treatment of refractory neurological diseases. Mesenchymal stromal cells (MSCs), one of the most important stem cells are highly proliferative, which have multilineage differentiation potential and can be obtained from various tissue sources, such as bone marrow (BM), adipose tissue and umbilical cord blood.

BM is the main source for MSCs isolation and BM-derived MSCs (BM-MSCs) have been studied extensively. However, BM harvesting is a highly invasive procedure for the donors and the proliferation efficiency, multipotent differentiation potential and maximal lifespan of BM-MSCs decline with age. MSCs can also be isolated from umbilical cord Wharton's jelly and differentiate into different types of cells, including adipocytes, osteocytes,
chondrocytes and neurons. Umbilical cord mesenchymal stem cells (UC-MSCs) are more primitiveMSCs than those isolated from other tissue sources and do not express the major histocompatibility complex (MHC) class II (HLA-DR) antigens. However, studies have shown that UC-MSCs are still viable and are not rejected 4 months after transplantation as xenografts without the need for immune suppression, suggesting that they are a favorable cell source for transplantation. Furthermore, umbilical cord can be easily obtained and represents a non-controversial source of MSCs. Therefore, UC-MSCs represent a promising alternative cell source for tissue engineering.

Evidence has shown that human umbilical cord mesenchymal stem cells (HUC-MSCs) can differentiate into many cell types, including neuron under appropriate conditions and have the potential to induce neurorestorative processes, including neurogenesis, angiogenesis and synaptic plasticity that are essential for facilitating recovery of neurological function. Therefore, transplantation of HUC-MSCs is a promising therapeutic strategy for the treatment of many neurological disorders. Studies have shown that HUC-MSCs therapy has been applied in many medically untreatable neurological conditions, such as spinal cord injury, cerebral palsy, post-apoplexy and traumatic brain syndrome, motor neuron disease and multiple sclerosis. However, the safety and efficacy of cell therapy depends on the mode of cell administration.

The development of a safe and effective strategy for cell transplantation has been a major clinical challenge in cell therapy. Previous studies have demonstrated that intravenous infusion of MSCs might be a feasible and safe mode for MSC treatment of stroke patients. However, several cells are distributed widely throughout the body, such as in the liver, spleen and kidneys. Furthermore, stem cell transplantation procedures using intraparenchymal injections have been reported to cause tissue injury, in addition to associated surgical risks. Intravenous cell administration gives engraftment in parenchymal lesions and the method has low efficacy and specificity. However, among various routes for HUC-MSCs infusion, intrathecal administration via the lumbar route is particularly challenging because of technical difficulties in this group of patients. Hence, in the present study, we have carried out a prospective analysis to assess the safety, therapeutic effect and the technical difficulties of HUC-MSCs intrathecal infusion in these patients.

Materials and Methods

Characteristics of participants

The one hundred patients with neurological disorders were recruited between December 2006 and May 2010 from the Affiliated Hospital of Medicine. There were 53 males and 47 females with a male to female ratio of 1.12:1. The age at diagnosis was in the range 2 to 68 yrs (median 40 yrs). In terms of diagnosis, 30 patients had spinal cord injury, 15 cerebral palsy, 15 post-traumatic brain syndrome, 8 post-brain infarction syndrome, 8 spinocerebellar ataxias and 12 motor neuron disease (Table 1). The local institutional review board of the Affiliated Hospital of Medicine, under the auspices of the National Ministry of Heath approved application of the technique and consent forms were obtained from each patient before initiation of treatment.

All patients were free of: i) Prior history of severe allergic reactions, ii) history of, or active, malignancy, iii) active systemic or severe focal infections (including HIV and syphilis), iv) active cardiac, pulmonary, renal, hepatic or gastrointestinal disease, v) coagulopathy or any other contraindication for lumbar puncture, vi) gastrostomy, tracheostomy or noninvasive ventilatory support as these could influence the prognosis and end-point measurements, vii) any severe psychiatric disorder, and viii) any immunodeficiency disease or condition.

As per protocol, the pre- and post-treatment study tested for complete blood counts, routine urine tests, liver function, renal function, electrolytes, sero-enzymology, blood glucose, blood lipids, cellular and humoral immunity, routine cerebro-spinal fluid (CSF) and biochemical markers (Biochemistry analyzer, Beckman, US and Epics-XL flow cytometer, Beckman, US) on the basis of previous studies.

Isolation of HUC-MSCs

The isolation, culture and expansion of the HUC-MSCs were performed according to the previously described method. In brief, human umbilical cord (HUC) was obtained from the Gynecology Department at the Affiliated Ren Ming Hospital of Hu Bei University of Medicine. Tissue collection for this study was approved by the affiliated Ren Ming Hospital Ethics Committee and informed consent was obtained from newborns’ parents. The tissue was minced into 1-2 mm3 pieces and incubated with
were measured from his/her L-spine MRI, one from an interval of 5 to 7 days. Two ml of cerebrospinal fluid depending on the patient’s condition within an interval of 5 to 7 days. Two ml of cerebrospinal fluid was removed and replaced by 2 ml of cell suspension. Cells from HUC were plated at a density of $1 \times 10^6$ cells/cm$^2$ in non-coated T-25 cell culture flasks (Becton Dickinson, San Jose, CA, USA). Growth medium consisted of Dulbecco’s modified Eagle’s medium with low glucose (Gibco, Grand Island, NY, USA) and 5% fetal bovine serum (FBS, HyClone, Logan, UT, USA), supplemented with 10 ng/ml vascular endothelial growth factor (Sigma), 10 ng/ml epidermal growth factor (Sigma), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma), and 2 mM/L glutamine (Gibco). Cultures were maintained in a humidified atmosphere with 5% CO$_2$ at 37°C. The medium was replaced and non-adherent cells were removed after 3 days. The medium was changed twice weekly thereafter. A cell monolayer formed within 2 weeks, consisting of homogeneous bipolar spindle-like cells in a whirlpool-like array.

Flow cytometric analysis showed that the HUC-derived cells were positive for CD29, CD44, CD105 and CD166, but negative for CD14, CD34, CD38, CD45, and HLA-DR. Once 60-80% confluence had been reached, adherent cells were replated at a density of $1 \times 10^4$ cm$^2$ in UC-MSCs growth medium (UC-GM) for expansion. After passage 3, cells were used for treatment. Flow cytometric analysis was performed on passage 2.

Cell administration

For each patient, two distances (depth) to the skin were measured from his/her L-spine MRI, one from the posterior margin of the spinal canal (minimal) and the other from the anterior margin of the spinal canal (maximal). Lumbar puncture was typically performed in the L4 and L5 interspace, while the patient was in the lateral position. HUC-MSCs were administered through intrathecal injection by lumbar puncture on the basis of previous studies.$^{22,23}$ Each patient received cell transplantation four to six times, depending on the patient’s condition within an interval of 5 to 7 days. Two ml of cerebrospinal fluid (CSF) was removed and replaced by 2 ml of cell suspension during the intrathecal injection. The needle was left in the same position for 5 min before withdrawal. Pediatric and uncooperative patients were given general anesthesia before performing lumbar puncture.

Technical difficulties in the form of localization of subarachnoid space, number of attempts, general anesthesia supplementation and post-procedural complications were evaluated and recorded. All patients were monitored in the wards for 24 h and hydrated with 3 L of fluid; ambulation was allowed 8 h post-procedure. Short- and long-term functional evaluation was done with Hauser ambulation index (HAI) by the HUC-MSCs transplant team on a regular basis.$^{24}$

Therapeutic effect criterion

There are no published criteria to measure therapeutic efficacy in the treatment of HUC-MSCs for neurological disorders. We applied HAI for the evaluation of treatment efficacy.$^{25}$ The HAI is a rating scale developed by Hauser et al. to assess mobility by evaluating the time and degree of assistance required to walk 25 feet. Scores range from 0 (asymptomatic and fully active) to 10 (bedridden), which 0 = Asymptomatic; fully active; 1 = Walks normally, but reports fatigue that interferes with athletic or other demanding activities; 2 = Abnormal gait or episodic imbalance; gait disorder is noticed by family and friends; able to walk 25 feet (8 m) in 10 s or less; 3 = Walks independently; able to walk 25 feet in 20 s or less; 4 = Requires unilateral support (cane or single crutch) to walk; walks 25 feet in 20 s or less; 5 = Requires bilateral support (canes, crutches, or walker) and walks 25 feet in 25 s or less; or requires unilateral support but needs more than 20 s to walk 25 feet; 6 = Requires bilateral support and more than 20 s to walk 25 feet; may use wheelchair on occasion; 7 = Walking limited to several steps with bilateral support; unable to walk 25 feet; may use wheelchair for most activities; 8 = Restricted to wheelchair; able to transfer self independently; 9 = Restricted to wheelchair; unable to transfer self independently. 10 = Bedridden.

Chemiluminescence intensity assay

The protocol for chemiluminescence measurements is described in previous study.$^{26}$ In brief, the wells of microtiter plates were filled with 100 μl of streptavidin solution (1 μg ml$^{-1}$) in PBS (pH = 7.4) and incubated overnight at 4°C. Next, the streptavidin solution was removed and the plates were post-coated with 200 μl of blocking solution for 1 h at room temperature. After washing four-times with 300 μl of washing solution, the microwells were filled with 100 μl of a diluted (1:500) biotinylated horse radish peroxidase (HRP) solution, after its elution
from a Sephadex G-25 column for 30 min at room temperature. Then, the wells were rewashed six-times using the same washing solution and chemiluminescence (CL) development was carried out by adding 150 μl of a freshly prepared luminol substrate solution (one for each enhancer) with a multi-channel pipette. The latter was consisted of luminol (0.10 mmol l–1), H2O2 (1.0 mmol l–1) and the enhancer in Tris buffer (0.10 M) with a pH value of 8.5. The optimum concentration for each enhancer was determined after performing a series of CL measurements and the major signal was observed at the same time respectively.

Results

Autonomic improvement of spinal cord injury

Administration of HUC-MSCs via intrathecal routes was well-tolerated during the clinical treatment course. Of 88 patients, we encountered technical difficulties in 20 patients (23%), of which 12 required general anesthesia supplementation, 3-needed Taylor’s approach and 5 required multiple attempts for localization of subarachnoid space. Ten patients had post-procedural headache, which was relieved within 24 h with analgesics, hydration, and rest; three had low-grade fever lasting for 24 h; three patients had low back pain and two patients had lower limb pain, which responded to within 24 h symptomatic treatment. On long-term follow-up, functional indices improved in 50 (31.67%) patients, including 15 patients with spinal cord injury, 10 with cerebral palsy, 10 with post-traumatic brain syndrome, 5 with post-brain infarction syndrome, 5 with spinocerebellar ataxias and 5 with motor neuron disease. Cerebral palsy and post-traumatic brain syndrome patients showed improvement in muscle tone, rigidity and spasm (Table 1).

Of the 30 spinal cord injury patients, 20 had previous history of spinal surgery. In 15 spinal cord injury patients with HAI scale improvement, 10 patients had an injury period of <1 yr, whereas 5 had injury period of >1 yr. Fifteen patients had improvement in motor power (10 with injury period of <1 yr and 5, >1 yr). Before treatment, all these patients had HAI grade 9. After HUC-MSCs transplantation, one grade 4, two grade 5, three grade 6 and four grade 7. Dramatic improvement was observed in four bed-ridden patients, who were able to walk with the help of a walker (HAI grade 9-HAI grade 4/5). In 12 patients, autonomic improvement was seen, including 8 patients who had <1 yr injury period. Of these, 3 became catheter-free and 2 required intermittent catheterization. Three patients had improvement in bowel sensations and sweating. A mixed motor and autonomic improvement as seen in 8 of 30 patients are shown in Table 2.

Testing of chemiluminescent intensity

Our results showed that activation of other two members of mitogen-activated protein kinase (MAPKs) — Jun N-terminal kinase (JNK) and p38 increased the rate of hepatocyte apoptosis, following ischemia-reperfusion (IR) injury. On the basis of these observations, we studied the effect of normothermic IR stress on the different members of the MAPK family. The expression of phospho-p44/42 ERK1/2 was significantly higher in the liver of animals treated with CTZ (Fig. 1) and this effect to UC-MSCs was significant.
Discussion

Stem cells, especially HUC-MSCs have been extensively cared due to their easily availability, expansion, faster proliferation and lower immunogenicity than BM-MSCs in animal experiments and preclinical studies, and may be a promising therapeutic strategy in neurological disorders. Studies have shown potential effect of UC-MSCs in the treatment of diseases in central nervous system (CNS), such as cerebrovascular disease, nerve degenerative disease, spinal cord injury, cerebral palsy, etc. The transplantation path in cell therapy is diversity, which mainly includes intervention path, local implantation, intravenous route and lumbar puncture way. Migration toward pathology is the first critical step in UC-MSCs engagement during regeneration, and it is hypothesized that the inflammatory response itself guides the behavior of potentially reparative UC-MSCs. Lieberman and colleagues found that introducing UC-MSCs into the subarachnoid space of the spinal cord transports the cells through CSF and allows more efficient delivery of cells to the injured area of CNS compared to the intravenous route.

Intrathecal UC-MSCs administration is safe, less invasive and a convenient procedure involving no surgery. However, the efficacy of UC-MSCs 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 UC-MSCs prior to transplantation.

We assessed the viability of UC-MSCs using 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 injecting the cells, the needle tip should be left in the same position for at least 5 min before withdrawing slowly to prevent any backflow.

Although UC-MSCs injection 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 spinal cord injury and 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, etc.\textsuperscript{34-36}.

Cerebral palsy patients need special attention because of multisystemic involvement making them American Society of Anesthesiologists (ASA) risk III patients. Cognitive, communication and behavioral problems along with co-existing diseases and drug therapy influenced the anesthetic management of some patients, as they had to be given general anesthesia. 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 post-operative period. After intraspinal injection of UC-MSCs in this patient population, some patients had post-procedural headache. This was probably due to many reasons, including the change of CSF, leakage of CSF and the use of a large-bore spinal needle, which was essential to prevent any mechanical damage to the cells during infusion. Three patients had low back pain and two patients had lower limb pain, probably due to nerve root injuries; however, they recovered early. All the side effects were resolved within 24 h with no long-term sequel.

The limitation of our study was that the study group was very heterogeneous in clinical presentation, duration of injury (4 months to 5 yrs), and follow-up time period, which was a major constraint in forming a clinically comparable control group. Review of other research studies has suggested multiple subcutaneous (SC) infusions, while we used a single infusion, which could have been another limiting factor. However, despite these limitations, our results were encouraging, as 61.7% of patients showed neurological function improvement. Further trials with clinically comparable groups are required to recommend use of UC-MSCs in neurological disorders.

In conclusion, this study suggests that subarachnoid placement of UC-MSCs 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.

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References
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