Cytotherapy, 2013; 15: 185–191

International Society for Cellular Therapy
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Clinical analysis of the treatment of spinal cord injury with umbilical cord mesenchymal stem cells

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Abstract

Background aims. The purpose of this study was to observe the clinical effect and safety of umbilical cord mesenchymal stem cells (UC-MSCs) in treating spinal cord injury (SCI) by intrathecal injection. *Methods.* From January 2008 to October 2010, we treated 22 patients with SCI with UC-MSCs by intrathecal injection; dosage was 1×10^6 cells/kg body weight once a week given four times as a course. Four patients received two courses, one patient received three courses and all other patients received one course. American Spinal Injury Association scoring system and International Association of Neurorestoratology Spinal Cord Injury Functional Rating Scale were used to evaluate neural function and ability to perform activities of daily living. *Results.* Treatment was effective in 13 of 22 patients; nine patients had no response. Among patients with incomplete SCI, the response to treatment was 81.25%; there was no response to treatment among six patients with complete SCI. Five patients with a response to treatment received two to three courses of therapy, and effects in these patients were further enhanced. In most patients in whom treatment was effective, motor or sensory functions, or both, were improved, and bowel and bladder control ability was improved. In 22 patients 1 month after therapy, algesia, tactile sensation, motion and activity of daily living scale were significantly improved ($P < 0.01$). During therapy, common adverse effects were headache (one case) and low back pain (one cases); these disappeared within 1–3 days. No treatment-related adverse events occurred during a follow-up period ranging from 3 months to 3 years. *Conclusions.* UC-MSC therapy by intrathecal injection is safe and can improve neurologic function and quality of life in most patients with incomplete SCI.

Key Words: *spinal cord injury, umbilical cord mesenchymal stem cells*

Introduction

Spinal cord injury (SCI) is a serious injury of the nervous system that can lead to paralysis and urinary and fecal incontinence. Several factors are thought to contribute to the lack of regeneration of spinal cord axons, including a reduction in the intrinsic growth capacity of adult central nervous system projection neurons, the presence of inhibitory cues derived from damaged central nervous system myelin, the formation of a glial scar by local astrocytes in response to inflammatory stimuli and the absence of neurotrophic factors and nerve growth factors. Neither medical nor physical therapy has shown a curative effect.

Many animal studies have shown that mesenchymal stem cells (MSCs) can promote the restoration of neurons. Investigators injected MSCs into the subarachnoid space of rats with spinal injury and found more MSCs in injury parts. Some MSCs can differentiate into neurons and neuroglial cells and promote the restoration of spinal injury (1,2). Yang *et al.* (3) transplanted human umbilical cord mesenchymal stem cells (UC-MSCs) into the lesion site of the rats with complete spinal cord transection. Significant improvements in locomotion were observed that were accompanied by increased numbers of regenerated axons in the corticospinal tract and neurofilament-positive fibers around the lesion site. Hypotheses as to why the MSCs can pass through the cerebrospinal fluid-brain barrier are as follows: (i) Various inflammatory factors and vasoactive substances are released after SCI, which can increase the permeability of the cerebrospinal fluid-brain barrier; (ii) MSCs have a chemotactic response to the injured parts of the spinal cord. Besides directly replacing damaged oligodendrocytes and neurons, MSCs could play an important supportive role in SCI therapies. They could create a more favorable environment for limiting damage and promoting regeneration, via immunoregulation, expression of

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(Received 25 February 2012; accepted 5 September 2012)

http://dx.doi.org/10.1016/j.jcyt.2012.09.005

growth factors and cytokines and improved vascularization, providing a permissive growth substrate or suppressing cavity formation, or both (4–6). These different mechanisms are not mutually exclusive, and numerous mechanisms could contribute to improved outcomes.

MSCs can be isolated from bone marrow (BM), umbilical cord (UC) and UC blood. The frequency of MSCs obtained from BM aspirates is about $\leq$0.01%. BM aspiration and *ex vivo* expansion of MSCs for about 3 weeks are needed to obtain MSCs from BM. Crucial points to isolate MSCs from UC blood are a time from collection to isolation, a net volume of UC blood and a mononuclear cell count. In 2003, Mitchell *et al.* (7) first identified MSCs from human UC Wharton's jelly. It was reported that human UC-MSCs have greater *ex vivo* expansion capabilities, faster proliferation and lower immunogenicity than bone marrow mesenchymal stem cells (BM-MSCs) (8,9). Human UC-MSCs can be induced to differentiate *in vitro* into bone, cartilage, adipose tissue (10,11), skeletal muscle cells (12), cardiomyocytes (13,14), endothelium (15) and especially neural cells (7,16–18). The cells possess a potential therapeutic role for treating patients with neurodegenerative diseases and central nervous system injuries. Shetty *et al.* (19) compared the MSCs from BM, UC and UC blood and found these cells all expressed CD73, CD105, SSEA4, CD29, CD44 and HLA-A, HLA-B and HLA-C. UC-MSCs and UC blood MSCs did not express HLA-DR associated with transplant rejection and could maintain the capacity of multilineage differentiation during *in vitro* culture. We used UC-MSCs in the present study. In this study, we observed the therapeutic effect of cell therapy with UC-MSCs by intrathecal injection in 22 patients with SCI.

Methods

Patients

We recruited 22 patients (17 men and five women, average age 33 years) with SCI at our hospital from January 2008 to October 2010. Four patients had cervical cord injuries, two patients had cervical cord and thoracic cord injuries, seven patients had thoracic cord injuries, two patients had thoracic cord and lumbar cord injuries and seven patients had lumbar cord injuries. The average time from injury to participation in the study was 56 months (range, 2–204 months). The causes of injury included motor vehicle accident (eight cases), a fall from a height (eight cases), a crush injury (2), diving accident (two cases), tethered cord syndrome (one case) and sequela of myelitis (one case). The risk of therapy with MSCs (e.g., fever, lumbago, headache, dizziness, suppressed cellular immunity, cerebral embolism, tumor, spasticity, neuropathic pain, possible loss of neurologic function) was explained to the patients, and informed consents were signed. The therapeutic regimen was approved by hospital Ethics Committee and Technology Committee. Before receiving MSC therapy, patients underwent a comprehensive evaluation including physical examination, electrocardiogram, magnetic resonance imaging of the spinal cord, complete blood count, biochemistry and hemostasis tests. No fever or infection was present in any of the patients, and all patients had normal function of the heart, lung, liver, kidney and hematologic system.

UC-MSC preparation

All parts of this study, especially the isolation of the human UC, were performed according to the Declaration of Helsinki. Ethical approval was obtained from the General Hospital of Air Force (Beijing, China), and written informed consent was obtained from UC donors. UC-MSCs were uniformly cultured in the Stem Cell Center of Air Force General Hospital. Cells from different UCs were sampled for immunophenotypic analysis and differentiation assays. MSC preparation was performed as previously reported (7). Briefly, UCs were collected after obtaining signed written informed consent and microbiologic detection. Each human UC was collected from full-term cesarean section births and processed within 3–6 h. Umbilical arteries and veins were removed, and the remaining tissue was diced into small fragments. Equal volume of 0.2% collagenase I was added, and the digestion was carried out at 37°C overnight. The next day, double volume of 0.05% trypsin was added and maintained for 1 h at 37°C. Cells were collected by centrifugation at 2000 rpm for 10 min. Viable cells were counted, suspended in human MSC serum-free culture media (HangZhou Biowish Bio-tech Co., HangZhou, China) and seeded into culture plastic flasks. A few colonies were formed after 7 days, and sub-cultivation was performed. The cells were passaged, and cells at passage 3 (about 21 days of culture) were used for therapy after phenotypic analysis with fluorescence-activated cell sorter (FACSCalibur flow cytometer; BD Biosciences, Franklin Lakes, NJ USA) and differentiation assays (Figures 1 and 2). Bacterial and fungal cultivation of the medium was performed 48 h before cell harvesting.

Clinical use of UC-MSCs

Lumbar punctures were performed after patients were admitted to the hospital. A dosage of 1×10^6/kg