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Human umbilical cord blood stem cell transplantation for the treatment of chronic spinal cord injury

Electrophysiological changes and long-term efficacy☆

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Abstract
Stem cell transplantation can promote functional restoration following acute spinal cord injury (injury time < 3 months), but the safety and long-term efficacy of this treatment need further exploration. In this study, 25 patients with traumatic spinal cord injury (injury time > 6 months) were treated with human umbilical cord blood stem cells *via* intravenous and intrathecal injection. The follow-up period was 12 months after transplantation. Results found that autonomic nerve functions were restored and the latent period of somatosensory evoked potentials was reduced. There were no severe adverse reactions in patients following stem cell transplantation. These experimental findings suggest that the transplantation of human umbilical cord blood stem cells is a safe and effective treatment for patients with traumatic spinal cord injury.

Key Words
neural regeneration; spinal cord injury; human umbilical cord blood stem cells; transplantation; paraplegia; American Spinal Cord Injury Association score; neurological function; secretion; somatosensory evoked potentials; spasm; safety; photographs-containing paper; neurogeneration

Research Highlights
(1) The safety of human umbilical cord blood stem cell transplantation in the treatment of traumatic spinal cord injury (injury time > 6 months) was observed.
(2) The restoration of neurological function was explored at 12 months after stem cell transplantation in patients with traumatic spinal cord injury. In addition, a neuroelectrophysiological monitoring system, the somatosensory evoked potential test, was performed to determine the nerve conduction functions of patients, thus reflecting the conduction of the spinal cord.

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INTRODUCTION

The existing methods of treatment for patients with traumatic spinal cord injury have limited effectiveness, which leads to an increased number of patients with neurological complications in the late stages of injury[1-5]. An attractive source for cell transplantation is human umbilical cord blood[6], and there have been several studies *in vitro* showing that umbilical cord blood cells secrete a number of cytokines that could be beneficial to recovery following spinal cord injury[7-9]. There are also a number of animal experiments that demonstrate the capacity of umbilical cord blood cells to differentiate into neural and glial cells[10-14]. These properties

are similar to multipotent mesenchymal cells found in bone marrow[11]. However, the conclusions of these studies are based on experimental animals, with rare studies of the safety and therapeutic effect of human umbilical cord blood stem cells in human being.

These studies concluded that intravenous injection is a safe approach and that infusion as close as possible to the injury site is the most effective[15-18]. Although these therapies were effective in the short-term, the long-term results of stem cell therapy have not been reported, and reports of the clinical application of human umbilical cord blood stem cells are rare. The aims of this study are to explore the long-term effect of human umbilical cord blood stem cell therapy, which combined intravenous injection and direct epidural injection. To evaluate the effect of stem cell therapy, we observed the patients' American Spinal Cord Injury Association score, autonomic nerve function, Ashworth scale, and somatosensory evoked potential value in limbs at different time points before and after treatment.

RESULTS

Quantitative analysis and baseline information of patients

Twenty-five patients with late-stage spinal cord injury (9 females and 16 males; injury time > 6 months) receiving human umbilical cord blood stem cell transplantation and traditional rehabilitation were chosen as the treatment group, and another 25 patients with late-stage spinal cord injury (10 females and 15 males) receiving only traditional rehabilitation served as the control group. Both groups were followed-up for 12 months after treatment (Table 1).

Table 1 Baseline analysis of involved patients in two groups

Group	Age (mean±SD, year)	Injury time (mean±SD, month)	Gender (M/F,*n*)
Treatment	36.7±3.8	11.4±3.6	16/9
Control	34.5±6.2	12.5±5.1	15/10

There were no significant differences in the average age and injury time between treatment and control groups ($P > 0.05$). $n = 25$. M: Male; F: female.

Complications of spinal cord injury patients after human umbilical cord blood stem cell transplantation

The stem cell treatment group comprised 9 females and 16 males, 5 (20%) of which were quadriplegic and 20 (80%) were paraplegic. Through regular and MRI examinations, there were no severe complications, neoplasm or aggravated neurological symptoms shown. Three (12%) patients had fever after infusion of stem cells; their body temperature was maintained at 37–38°C, there were no abnormities in the levels of white blood cells and the fever was retained for less than 24 hours. The fever could be controlled by physical hypothermia. There was no statistically significant difference in terms of complication rates between the paraplegic and the quadriplegic patients ($P > 0.05$).

Improvements of neurological function in patients with spinal cord injury after human umbilical cord blood stem cell transplantation (Table 2)

Table 2 The amount and percentage of patients [*n* (%)] with improvements in different functions after human umbilical cord blood stem cell transplantation

Index	Spinal cord injury			Total
	Cervical	Thoracic	Lumbar	
Improvement in ASIA score	1(4)	2(8)	1(4)	4(16)
Decrease in spasm	3(12)	4(16)	0(0)	7(28)
Improvement in autonomic function	2(8)	4(16)	2(8)	8(32)
Improvement in urinary function	1(4)	3(12)	2(8)	6(24)
Improvement in SSEP test	2(8)	5(20)	2(8)	9(36)

The ratio of patients was calculated and expressed by percentage (%), $n = 25$. Evaluation standard of ASIA score complied with the International Standards for Neurological Classification of Spinal Cord Injury. ASIA: American Spinal Cord Injury Association; SSEP: somatosensory evoked potential.

At 12 months after stem cell therapy, 4 patients (16 %) showed improvements in American Spinal Cord Injury Association score: one case was cervical spinal cord injury, two cases were thoracic spinal cord injury, and one case was lumbar spinal cord injury. Spasm decreased in seven patients (28%) after stem cell therapy, including three cases with cervical spinal cord injury and four cases with thoracic spinal cord injury. Eight patients (32%) had improved autonomic function after stem cell therapy, including two cases with cervical spinal cord injury, four cases with thoracic spinal cord injury, and two cases with lumbar spinal cord injury. Six patients (24%) had improved urinary function after stem cell therapy, including one case with cervical spinal cord injury, three cases with thoracic spinal cord injury, and two cases with lumbar spinal cord injury (Table 2, Figure 1). Nine patients (36%) had improved somatosensory evoked potential tests after stem cell therapy, including two cases with cervical spinal cord

injury, five cases with thoracic spinal cord injury, and two cases with lumbar spinal cord injury (Table 2).

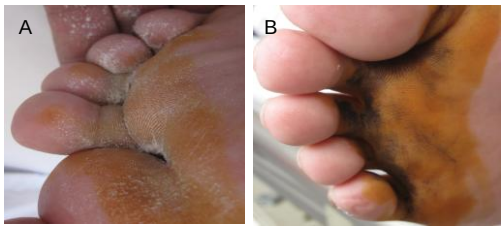

Figure 1 The improvement of sweating test results before and after stem cell therapy.

Images are of a male patient with traumatic spinal cord injury, 35 years old, injury time > 6 months and injury level was L_1. The patient lost his sweating function before treatment, the negative result in sweating test indicated a loss in motor function, sensation and sweating function (A). At 6 months after treatment, a positive result in sweating test in the same patient indicated recovery of sweating function (B).

Compared with the stage before stem cell therapy, no statistically significant difference in American Spinal Cord Injury Association score was found after stem cell therapy ($P > 0.05$; Table 3).

Table 3 The American Spinal Cord Injury Association (ASIA) score before and after stem cell treatment

Index	Pretreatment	After treatment	
		6 months	12 months
ASIA sensory score	100.2±21.1	103.1±8.3	105.0±11.4
ASIA motor score	33.5±11.6	34.3±14.2	35.2±9.1

A total sensory score of 224 relates to normal sensory function of different key points in every level. Evaluation standard: For each key point, normal sensory function – score 2; abnormal sensory function – score 1; no sensory function – score 0. The total score of all key points in body is the ASIA score. A total motor score of 100 relates to normal motor function, with strength in different key muscles. The higher score was related to the patients' better sensory and motor function.

There was no statistically significant difference ($P > 0.05$) of ASIA sensory and motor score at different time points in the stem cell treatment group. Data were calculated and expressed as mean±SD, $n = 25$, Student's t-test (data were in accordance with normal distribution) was applied to two non-related parametric samples (independent or non-paired). There was no statistically significant difference ($P > 0.05$) of ASIA and motor score between different time points in the stem cell treatment group.

Improvements in somatosensory evoked potential test after human umbilical cord blood stem cell transplantaion

Nine cases (36%) showed a positive response in somatosensory evoked potential (Table 2). After stem cell therapy, there were statistically significantly differences in latency time (milliseconds), which were measured by evoked potentials (p40Fcortex) of the right and left lower limbs compared with the stage before therapy ($P < 0.05$; Table 4, Figure 2). In those patients who showed improvement in somatosensory evoked potential, there was a mean time of 6 months between infusion and lower limb improvement.

Table 4 Somatosensory evoked potentials measured by evoked potentials (p40Fcortex) of the right and left lower limbs after human umbilical cord blood stem cell transplantation

Item	Treatment group	Control group
Pre-treatment		
Right	69.1±14.2	72.6±13.1
Left	58.1±17.7	65.2±15.4
6 months after treatment		
Right	59.2±9.4[ac]	69.9±11.4
Left	53.2±15.6[ac]	64.6±14.7
12 months after treatment		
Right	55.6±15.8[bc]	68.5±15.2
Left	50.4±16.8[bc]	65.8±9.9

The somatosensory evoked potentials were measured by evoked potentials (p40Fcortex) and expressed by latency (ms), intergroup comparisons were applied for the mean estimates.

[a]$P < 0.05$, *vs*. pre-treatment in the same lateral limbs. [b]$P < 0.05$, *vs*. 6 months after treatment in the same lateral limbs. [c]$P < 0.05$, *vs*. control group. The data were calculated and expressed by mean±SD, $n = 25$, Student's t-test was applied to paired samples or two non-related parametric samples (independent or non-paired).

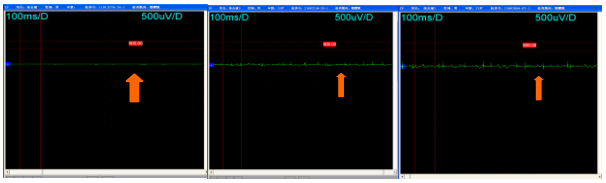

Figure 2 Results of somatosensory evoked potentials (SSEP) test in lower limbs at different time points before and after stem cell therapy.

The orange arrows revealed the change in shape of the SSEP wave at different time points. Distinct improvements in SSEP results was found at different time points after stem cell trerapy. Before stem cell therapy, the latency time was at a high level (left); 6 months after stem cell therapy, the latency time (s) was decreased (middle); 12 months after therapy, there was a statistically significant difference compared with both 6 and 12 months (right).

DISCUSSION

Nowadays, there are no effective therapies for spinal cord injury because of the limited spontaneous endogenous regeneration of damaged/lost oligodendrocytes in the spinal cord[19]. Potential strategies to treat spinal cord injury could be aimed at promoting remyelination *via* oligodendrocyte transplantation[20], controlling apoptosis, and promoting endogenous regeneration of dead cells[21]. In previous studies, human umbilical cord blood stem cells have

been shown to have the following properties: they have the potential to differentiate *in vitro* into cells that are morphologically similar to oligodendrocytes and express oligodendrocyte markers; they secrete factors that prevent further injury; have tropism for the injured area in the spinal cord; can be effective even through remote infusion either by intravascular or intrathecal administration; and improve neurological function in animal studies[22-24]. Therefore, the clinical application of human umbilical cord blood stem cells to treat spinal cord injury is very appealing. They concluded that intravenous injection is a safe approach and infusion applied as close as possible to the injury site provides the best results[25-26]. However, reports on the clinical application of human umbilical cord blood stem cells, which are easier to acquire and cultivate are rare. Our study supplies some important information relating to the safety and effects of human umbilical cord blood stem cells in patients with spinal cord injury.

The study samples were homogeneous as we chose patients in the late stages of traumatic spinal cord injury, which is the most widely studied and well-established condition[27-28]. Improvements in neurological function and somatosensory evoked potential tests were observed in some patients. There was no neoplasm or aggravated neurological symptoms after human umbilical cord blood stem cell therapy. Three patients had low-grade fever shortly (< 24 hours) after infusion of stem cells, but they had no abnormities in the blood or cerebral spinal fluid. One of the patients with low-grade fever showed improvement in autonomic function, so we considered the fever was a slight immune reaction, which had no influence on the safety and therapeutic effect of the treatment. The causes and effects of fever reaction need further exploration. Eleven cases (44%) showed improved neurological function in different aspects; the majority (40%) improved in autonomic neurological function (sweating function or bladder function), nine cases (36%) improved in electrophysiology test, and four cases (16%) improved in the American Spinal Cord Injury Association score. The improvement in neurological function and cortical response to peripheral stimuli may be explained by the formation of new synapses between host neurons and neurons formed after stem cell transplantation[29], or by newly myelinated glial cells derived from the transplanted stem cells[30-31].

The results of this study are very appealing as human umbilical cord blood stem cells are easy to acquire and its safety and effects were confirmed. The subjects of the study were patients with spinal cord injury for at least 6 months, as it is well-known that neurological recovery in patients with spinal cord injury largely occurs in the first 6 months post-injury[32]. Hence, any change in the neurological status of these patients could be attributed to the therapeutic approach under investigation. This study confirmed the safety and effects of stem cell therapy in the primary trial. Human umbilical blood stem cell treatment in patients with traumatic spinal cord injury in late stages resulted in improvement of neurological status, and confirmed the safety and therapeutic effects of human umbilical blood stem cells.

MATERIALS AND METHODS

Design

A clinical retrospective study.

Time and setting

The study was accomplished from July 2010 to March 2011 in the Second Affiliated Hospital of Kunming Medical University, China.

Subjects

Twenty-five patients with spinal cord injury in the late stage (injury time > 6 months) were included as the treatment group, aged 18–48 years.

All cases had complete or incomplete traumatic cervical, thoracic and lumbar injury. Cases with primary spinal cord disease, such as myelitis, infection and tumor, were excluded.

Another 25 patients with spinal cord injury in the late stage (injury time > 6 months), aged over 18 years, who received only traditional rehabilitation therapy and no stem cell therapy were included as the control group. The inclusion and exclusion criteria were the same as treatment group.

Before receiving rehabilitation, all patients underwent spinal surgery in the orthopedics department of different hospitals. All patients had normal blood cell counts and had no tumor or coagulation disorders. There were 5 cases of cervical spinal injury, 11 cases of thoracic spinal cord injury and 9 cases of lumbar spinal cord injury. No patient had shown any neurological improvement before stem cell therapy. All patients received somatosensory evoked potentials tests[18] and other tests of neurological function before and after the stem cell therapy. We examined the sensation and muscle strength in key points of bilateral limbs, and used the American Spinal

Cord Injury Association score to evaluate the sensory and motor function of patients. All patients were followed-up for 12 months after stem cell therapy. The moral principles of this study were in accordance with the Administrative Regulations of Medical Institutions formulated by the State Council of the People's Republic of China[33].

Methods

Preparation of human umbilical cord blood stem cells

Umbilical cord blood (100–150 mL) was collected from healthy unrelated donors, after obtaining signed informed consent forms in accordance with the sterile procurement guidelines for cord blood in each hospital[34]. Mononuclear cells were collected and washed twice in saline. Contaminating erythrocytes were lysed with lysis buffer (Beyotime, Shanghai, China) comprising of injection-grade water. Cell density was adjusted to 2–6 × 10^6/mL and seeded in DMEM/F12 culture medium with basic fibroblast growth factor and epidermal growth factor (Peprotech, Rocky Hill, NJ, USA) at a concentration of 20 ng/mL. Culture media (DMEM/F12; Gibco, New York, USA) was mixed with 2% v/v B-27 Stem Cell Culture Supplement (Gibco). Cells were cultured at 37°C with saturated humidity and 5% CO_2 by volume. At this stage, all relevant information about the initial culture was entered in the batch information record, including test results for sterility, mycoplasma and endotoxins. Cell growth was regularly monitored and the inspection records were updated accordingly. Cells were harvested for clinical application after 1 week of cultivation with cell quantity ≥ 1 × 10^7 and viability ≥ 95%.

To ensure the quality of the umbilical cord blood-derived mononuclear cells, a number of parameters were confirmed before use. Raw material control: Tests for communicable diseases (hepatitis B virus, hepatitis C virus, human immunodeficiency virus, alanine transaminase and syphilis) for umbilical cord blood units were performed before any processing began. Testing was performed by a third party laboratory under local government-monitored conditions.

In-process control: Non-qualifying cells were eliminated in accordance with Beike's cell counting and morphology standards, which include a cell quantity of ≥ 1 × 10^7 and highly homogeneous cells that have a rounded shape and have detached from the culture flask.

Culture control: Any contaminated cell suspensions or unhealthy cells were eliminated upon discovery. Contamination was determined by the presence of mycoplasma or visible microorganisms by microscopy. Furthermore samples were required to have an endotoxin level ≤ 0.5 EU/mL and be negative of free DNA.

Finished product control: This incorporates a final cell count (≥ 1 × 10^7), containing 1.0–2.0% $CD34^+$ cells as determined by flow cytometry (BD Bioscience Pharmingen Inc., San Diego, CA, USA), cell viability (≥ 95%) and sterility test.

Transplantation of human umbilical cord blood stem cells

Depending on the patient's condition, they were admitted to receive stem cell infusion by lumbar puncture and intravenous infusion, which was repeated four or five times. Treatments were separated by 1 week intervals. At the first time of therapy, a 30-mL intravenous infusion of cell suspension was administered through an intravenous catheter over a period of 20–30 minutes. Following this, the next three treatments were administered by lumbar puncture, which was performed in the lateral decubitus position, with the patient prepped and draped in sterile fashion, and the needle placed in the lumbar subarachnoid space. Flow of the cerebrospinal fluid into the syringe needle was evidence of the needle being in the correct place in subarachnoid space. Thus, the stem cells could be injected into the correct place successfully and accordingly exert their effects, which was the criterion of successful stem cell transplantation. 4 mL of cerebrospinal fluid was removed and replaced with 4 mL of cell suspension containing 1–3 × 10^7 cells. The color and pressure of the cerebrospinal fluid were observed and recorded to determine whether they were normal. During the progress, any abnormal reactions of the patients were observed. Stem cell therapy was implemented by Professor Ao, who was the item director of stem cell treatment for spinal cord injury.

Before receiving traditional rehabilitation, such as strength exercise and electrical stimulation, all patients received spinal fixation surgery by hospital orthopedic departments. Then, the patients in the treatment and control groups received their corresponding treatments between July 2010 and March 2011 in the Second Affiliated Hospital of Kunming Medical University, China. To evaluate the effect of stem cell therapy, the patients were informed to return to the hospital for functional evaluation at different time points during follow-up assessments.

Observation during 12-month follow-up after stem cell therapy

From the first day after infusion, abnormal reactions, such as fever, headache or lumbago, were recorded and the patients received rehabilitation training at an early stage if they had no abnormal reactions. The safety was evaluated by patient complication rates. The neurological functions were evaluated by American Spinal Cord Injury Association score[34], which evaluates the strength of 10 symmetrical muscle groups and different sensory levels in the body, and scores them as follows: A: complete injury; B: sensory function remains, but the motor function is lost; C: sensory and motor function remains, but more than half of the muscle groups have a strength < 3 below the injury level; D: sensory and motor function remains, but more than half of muscle groups have a strength > 3 below the injury level; E: normal function.

Other standards that were evaluated included autonomic nerve function (sweating), Ashworth scales, and somatosensory evoked potential values in limbs at different time points before and after treatment[18]. Sweating tests were performed using dry iodine and amylum. Dry iodine and dry amylum were placed on the skin of the patients' toe; if the patient had normal sweating function, the iodine and amylum would become wet and the amylum would change color from white to blue. The somatosensory evoked potential tests were detected using an electromyogram instrument (NTS-2000; Nuo Cheng, Shanghai, China). The results of this study were conducted and evaluated by the same doctor. The positive effects of stem cell therapy were defined by improvement of American Spinal Cord Injury Association score (from A to E), improvement of autonomic nerve function (revival of sweating function), decreased spasm (Ashworth score from 5 to 0) and revival of neurological transmit function, which was indicated by a reduced response time(s) of lower limb(s) under stimulation in both paraplegic and quadriplegic patients after treatment.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 statistical software (SPSS, Chicago, IL, USA). Rates of complication and effectiveness were calculated and expressed as percentage. Statistical data were expressed as mean ± SD, and intergroup comparisons were applied for the mean estimates, Student's *t*-test (the data was in accordance with normal distribution) was applied to two non-related parametric samples (independent or non-paired). A level of 5% was set as significant.

Author contributions: Liqing Yao was responsible for study design, data analysis, and paper writing. Lijuan Ao was responsible for study design and paper revision. Chuan He, Ying Zhao, Jirong Wang, Mei Tang, Jun Li, Ying Wu and Xiang He helped with data collection, stem cell therapy and follow-up. All authors read the paper and agreed to the publication.

Conflicts of interest: None declared.

Ethical approval: This study was approved by Research Ethics Committee of the Second Affiliated Hospital of Kunming Medical University in China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application disputations.

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