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Autologous Bone Marrow Mesenchymal Stem Cell Therapy in the Subacute Stage of Traumatic Brain Injury by Lumbar Puncture

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Abstract

Objectives: **To explore the clinical therapeutic effects and safety of autologous bone marrow mesenchymal stem cell therapy for traumatic brain injury by lumbar puncture.**

Materials and Methods: **A total of 97 patients (24 with persistent vegetative state and 73 with disturbance motor activity) who developed a complex cerebral lesion after traumatic brain injury received autologous bone marrow mesenchymal stem cell therapy voluntarily. The stem cells were isolated from the bone marrow of the patients and transplanted into the subarachnoid space by lumbar puncture.**

Results: **Fourteen days after cell therapy, no serious complications or adverse events were reported. To a certain extent, 38 of 97 patients (39.2%) improved in the function of brain after transplant (*P* = .007). Eleven of 24 patients (45.8%) with persistent vegetative state showed posttherapeutic improvements in consciousness (*P* = .024). Twenty-seven of 73 patients (37.0%) with a motor disorder began to show improvements in motor functions (*P* = .025). The age of patients and the time elapsed between injury and therapy had effects on the outcomes of the cellular therapy (*P* < .05). No correlation was found between the number of cell injections and improvements (*P* > .05).**

Conclusions: **This study suggests that the bone marrow stem cell therapy is safe and effective on patients with traumatic brain injury complications, such as persistent vegetative state and motor disorder, through lumbar puncture. Young patients improve more easily than older ones. The earlier the cellular therapy begins in the subacute stage of traumatic brain injury, the better the results.**

Key words: *Cerebral lesion, Regenerative therapy, Transplant, Vegetative state, Motor.*

Introduction

It is well known that traumatic brain injury (TBI) is a major public health problem worldwide. Traumatic brain injury is the No. 1 cause of coma and the leading role in disability in children and young adults.[1] Recently, prehospital and intensive care of patients with TBI has improved substantially, and evidence-based guidelines for management have been developed.[2] However, the prognosis for patients with severe TBI remains poor, such as disturbance of consciousness and motor disorder. Even under the best circumstances, mortality for acute severe TBI is around 36%, it is 15% for severe disability, it is 20% for moderate disability, and 25% for complete recovery.[3] Because the regeneration capacity of neurons is low, most patients' recovery will have occurred within 6 months of the injury, although further, slower improvement may occur in the next 12 to 18 months.[4] Therefore, it is ongoing that transplanting stem cells to the cerebral lesion area and inducing them to differentiate to neurons substitute neuronal function.

Stem cells are classically defined as *cells that have the ability to renew themselves continuously and possess*

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multipotent ability to differentiate into many cell types.[5] Human bone marrow mesenchymal stem cells (BMSCs) have been widely studied because of their relative easy access and differentiation potential to the osteogenic, adipogenic, chondrogenic lineages, hepatocytes, cardiomyocytes, neurons, and other kinds of tissues or cells.[6] Their multipotentiality and self-renewal have increased the attention to this stem cell model as a self-renewing cell source, with applications in tissue engineering and regenerative medicine.[7] For example, the osteogenic potential of BMSCs has been explored extensively in the biological evaluation of bone tissue engineering scaffolding structures.[8,9] Furthermore, several studies have reported that transplanted BMSCs accelerate neuroplasticity and facilitate neuronal regeneration, as well as functional recovery.[10-14]

Therefore, BMSC therapy may be a novel method to repair brain lesions and promote patients with functional disorders after severe TBI. Here, we report our experiences with autologous BMSC transplant that we have used in a clinical trial for patients with TBI complications.

Materials and Methods

Introduction of patients

The study was approved by and registered by the ethical committee of the Hospital and Health Bureau of City and patients gave their informed consent. The research reported in the paper was undertaken in compliance with the 1975 Helsinki Declaration and the International Principles. Forty-five patients who presented with a vegetative state and 121 patients who showed disturbance in their motor activity after severe TBI for at least 1 month were admitted to the department of neurosurgery. These patients had a diagnosis of severe TBI based on clinical evidence and neuroimaging, most prominently a radiologic test, for example computed tomography and magnetic resonance imaging. And these patients did not have other serious complications (eg, cachexia, pulmonary infection, and gastrointestinal bleeding). Ninety-seven patients (24 patients with vegetative state and 73 patients with disturbance motor activities) received BMSC transplant voluntarily. All the patients in the trial had been stabilized before the cell therapy with no apparent improvements in their motor activities and consciousness. Before regular investigations and therapy, the blood routines and serum biochemical indexes of these patients were checked to exclude inflammation, liver and renal insufficiency, and blood diseases. The study is a nonrandom, open-labeled, interventional cohort study.

Bone marrow mesenchymal stem cell recovery

The biological material used in this study would have been otherwise discarded during a standard surgical procedure. The procedure of isolating BMSCs refer to the steps reported in the studies.[15,16] About 100 mL of bone marrow was recovered by multiple aspirations in the posterior iliac crests in a heparinized (1 mL/5000 U) bottle and diluted in Dulbecco's phosphate buffered saline (without calcium and magnesium) at a ratio of 1:2. This was performed under sterile conditions with local anesthesia in the operating suite. The obtained solution was collected and filtered with a 70-μm cell strainer (Falcon, Pittsburgh, PA, USA) before centrifugation at 400 *g* for 10 minutes. The cell interface was carefully removed, and washed twice in Dulbecco's phosphate buffered saline at 400 *g* for 10 minutes. The resultant pellet was added with red blood cell lysing solution (0.7% ammonium chloride) and incubated for 2 minutes. Lysing was arrested by adding 0.9% ice-cold sodium chloride, and the cells were washed in Dulbecco's phosphate buffered saline until the lysing factors were removed. Cell pellets were resuspended in Dulbecco's modified Eagle's Medium (Sigma, St. Louis, MO, USA), supplemented with 10% Fetal Bovine Serum (Invitrogen Corporation, Carlsbad, CA, USA) and 1% antibiotics (streptomycin and penicillin) (Invitrogen), and cultured in 25-cm^2 flasks at 37°C in a humidified atmosphere containing 5% CO_2. On day 4, the cultures were washed with phosphate buffered saline to remove the nonadherent cells. Finally, cell pellets were resuspended in 5 mL (about 1×10^6 cells/mL).

Identification of bone marrow stem cells

The International Society for Cellular Therapy proposed a set of minimal criteria for the characterization of BMSCs, which includes the capability of adherence to plastic surfaces and the expression of the cell surface markers CD73, CD90, and CD105.[17] In this study, BMSCs were identified by examining the markers above as the fluorescence-activated cell sorting characterization of the stem

cells. About 100-μL cell samples were incubated with CD73, CD90, and CD105 antibodies conjugated with PE and FITC (BD Biosciences, San Jose, CA, USA) at a concentration of 2 μg/mL for 15 minutes at room temperature in the dark. After that, 1 mL of phosphate buffered saline was added to the stained cells and mixed well. Then, 5 μL of the 7-aminoactinomycin D dye was added and again incubated in the dark for 10 minutes at room temperature. The cells were analyzed by flow cytometry.

Installation of bone marrow stem cells

Ninety-seven patients decided to receive stem cell therapy by lumbar puncture. First, we made sure that the localized bacterial infection of the pars lumbalis skin did not exist before lumbar puncture. The patients had been subjected to local anesthesia. The BMSCs suspension (5 mL) was installed into the subarachnoid space by lumbar puncture between the lumbar vertebrae L3/L4 or L4/L5. Cell suspension was slowly injected into the subarachnoid space for 2 to 3 minutes. Then, patients were maintained in a supine position for 24 to 48 hours. Fourteen days after therapy, the patients were followed-up for scheduled examinations.

Persistent vegetative state evaluation

The vegetative state is a clinical condition of complete unawareness of the self and the environment accompanied by sleep-wake cycles with either complete or partial preservation of hypothalamic and brainstem autonomic functions. The vegetative state can be diagnosed according to the criteria described in the book.[18] The persistent vegetative state (PVS) can be defined as *a vegetative state present at 1 month after acute traumatic or nontraumatic brain injury, and present for at least 1 month in degenerative/metabolic disorders or developmental malformations*. In this study, improvements of PVS patients were evaluated according to the grade principle of PVS (drafted by Chinese Medical Association in Nanjing, China, 1996).

Motor function evaluation

Most motor disorders lack a distinctive biomarker, so evaluation of motor disorders is primarily clinical, based on careful neurologic examination. The clinical features of traumatic motor disorders include: (1) increased tone of clasp-knife type, (2) weakness most evident in antigravity muscles, (3) increased reflexes and clonus, (4) shocklike contractions of muscles, and (5) uncoordinated muscle movements.

Statistical Analyses

The chi-square, Fisher exact test, and 1-way analysis of variance were performed to analyze the data. All tests were considered significant at P values that were less than .05. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 12.0, IBM Corporation, Armonk, New York, USA).

Results

Safety of cell therapy

Although 5 patients expressed transient fever, and 2 patients felt light headache on second day after the cellular therapy, no patient experienced any serious adverse event (such as inflammation, systemic infections, and gastrointestinal bleeding) upon BMSCs reinfusion. No complications or wound infections were observed in the patients after the cellular therapy.

Results of cellular therapy

Fourteen days after cellular therapy, 38 of 97 patients (39.2%) with TBI improved (P = .007) (Table 1). Eleven patients begin to considerably show posttherapeutic improvements in consciousness (P = .024) (Table 2); they expressed responsive eyeball tracking occasionally, groaning, or tearing. Twenty-seven of 73 patients (37.0%) showed improvements in motor functions (P = .025) (Table 3). The patients with hemiplegic paralysis showed motor power and scale enhanced after the cellular therapy. The patients with muscle spasticity expressed muscular tension relaxed partly. The age of patients influenced the outcome of the cellular therapy (P < .05) (Tables 4 and 5), and the time elapsed between injury and therapy had roles on the outcome of the therapy on motor disorder (P < .05)

Table 1. The Results of Cell Therapy on TBI (Case)

Groups	Improvement	No improvement	Total
Therapeutic group	38	59	97
Nontherapeutic group	14	55	69
Total	52	114	166

Abbreviations: TBI, traumatic brain injury

(Tables 4 and 5). No correlation was found between the number of cell injections and improvement ($P > .05$) (Tables 4 and 5).

Table 2. The Improvements on Posttraumatic PVS After Cell Therapy (Case)

Groups	Therapeutic Group	Nontherapeutic Group	Total
Improvement			
Responsive eyeball tracking	3	1	4
Groaning	1	0	1
Responsive tearing	5	1	6
Swallow fluid food	2	1	3
No improvement	13	18	31
Total	24	21	45

Abbreviations: PVS, persistent vegetative state

Table 3. Improvements on Motor Disorder After Cell Therapy (Case)

Groups	Therapeutic Group	Nontherapeutic Group	Total
Improvement			
Clasp-knife type relaxed	6	2	8
Muscle power enhanced	14	5	19
Reflexes and clonus relaxed	4	1	5
Uncoordinated muscle movements reduced	3	1	4
No improvement	46	39	85
Total	73	48	121

Table 4. PVS After TBI: Group Assessment

Patient Data	Conclusions	n	Mean	SD	*P* Value
Age (y)	Improvement	11	21.1	3.47720	.012
	No improvement	13	35.3	8.36047	
Number of cells injection	Improvement	11	4.32×10^6	1.9872×10^6	.122
	No improvement	13	3.92×10^6	1.3259×10^6	
Time elapsed between injury and therapy (mo)	Improvement	11	1.51	0.24837	.006
	No improvement	13	2.52	0.50718	

Abbreviations: PVS, persistent vegetative state; TBI, traumatic brain injury

Table 5. Motor Disorder After TBI: Group Assessment

Patient Data	Conclusions	n	Mean	SD	*P* Value
Age (y)	Improvement	27	23.5	5.49242	.008
	No improvement	46	38.2	11.76897	
Number of cells injection	Improvement	27	4.12×10^6	2.5634×10^6	.116
	No improvement	46	3.63×10^6	1.4376×10^6	
Time elapsed between injury and therapy (mo)	Improvement	27	1.35	0.32054	.013
	No improvement	46	2.87	0.65786	

Discussion

All severity levels of TBI have the potential to cause significant, long-lasting disability.[19] Permanent disability is thought to occur in 100% of severe injuries.[20] Prognosis worsens with the severity and location of the lesion and depends on the access to immediate, effective acute management.[21] It is important to begin emergency treatment within the acute stage. In the subacute stage, prognosis is strongly affected by the patient's involvement in activity that promotes recovery.[1,2] The results of TBI vary widely in type and duration; they include physical, cognitive, emotional, and behavioral complications. Traumatic brain injury can cause prolonged or permanent influences on consciousness, for example PVS. Movement disorders that may develop after TBI include tremor, ataxia (uncoordinated muscle movements), myoclonus (shocklike contractions of muscles), and loss of movement range and control (in particular with a loss of movement repertoire).[22]

Loss of cellular components and myelination that occur as an inflammatory process hamper functional recovery.[15] Therefore, reducing progressive tissue damage and scarring, facilitation of remyelination, and re-establishment of lost neural tissue and its circuitry should be addressed for any successful cellular therapy. Bone marrow stem cells are multipotent adult progenitor cells that can differentiate into a variety of cell lineages,[23] which make BMSCs excellent candidates as therapeutic cells for the repair of damaged tissue. In the case of severe tissue damage, BMSCs can be attracted to the damaged sites.[24] The migration of BMSCs to injury sites, where they secrete bioactive factors that trophically influence the repair and regenerative process, produce factors that inhibit scarring and apoptosis, promote angiogenesis and stimulate host progenitors to divide, and differentiate into neurons and astrocytes to repair the injured tissue, leading to improved function.[25,26] Further, magnetic resonance imaging volumetric data reveal no significant change in grey matter, white matter, intracranial volume, or cerebral spinal fluid space at 1 and 6 months as measured related to expected norms.[27] In this regard, the positive effects of BMSCs may have important clinical use.

In this study, we isolated BMSCs from the bone marrow of the patients themselves and transplanted stem cells back into the patients. Excitingly, 14 days after BMSC therapy, 38 of 97 patients who received the stem cell therapy had improvements. The mechanisms of potential therapeutic benefit of

BMSC therapy may be as follows[28]: the secretion of growth factors, the exchange of genes and proteins through cell-to-cell fusion or contact, the induction of angiogenesis, and the effects on immune modulation. However, there have been numerous conflicting reports regarding stem cell engraftment and therapeutic efficiency. In this study, 59 of 97 patients who received the stem cell therapy did not improve. The reasons might be related to the effects of media, cell passage number/techniques, or isolation methods.[28] The authors also found that BMSC therapy had more-definite effects on younger patients. The probable reason was that young patients were in better body basal condition or bone marrow condition than older ones. Our results revealed an inverse relation between the time elapsed after the injury and the outcome of cellular therapy. Maybe 1 reason for this is that scar tissue forms in the damaged site after a longer elapsed time after the injury and stops migration of stem cells.

Additionally, increasing amounts of research are being conducted to evaluate multiple routes for delivery of stem cells, such as intravenous, intra-arterial, and direct routes. Intravenous administration offers easy access to the circulation, with the possibility of distribution throughout multiple tissues.[29] An initial drawback of intravenous application is the large proportion of first-pass pulmonary sequestration.[30] Intra-arterial administration offers a method to further localize the placement of stem cells. However, recent investigation has shown that intracarotid infusion of MSCs induces ischemic stroke.[31] Direct or intracerebral implantation of stem cells would maximize the stem cell load at the site of injury.[32] But investigators must consider the invasiveness of the intracerebral approach and the possibility of further tissue damage during cell transplant. Lumbar puncture delivery of BMSCs appears to be superior to other methods. Cell engraftment and tissue sparing were significantly better after lumbar puncture delivery, and host immune response after lumbar puncture delivery was reduced.[33] When BMSCs were introduced through a lumbar puncture, stem cells prevented astrogliosis and microglial activation, and spared and regenerated motoneurons.[34] In this study, we performed BMSCs transplant by lumbar puncture and no complications appeared.

Conclusions

This study suggests that BMSC therapy is safe and effective on patients with severe TBI complications, such as PVS and motor disorder, through lumbar puncture. Young patients improve more than older ones do. The earlier the cellular therapy begins in the subacute stage of traumatic brain injury, the better the results.

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