Application of Autologous Bone Marrow Stem Cells in the Therapy of Spinal Cord Injury Patients

E. R. Chernykh, V. V. Stupak, G. M. Muradov, M. Yu. Sizikov, E. Ya. Shevela, O. Yu. Leplina, M. A. Tikhonova, A. D. Kulagin, I. A. Lisukov, A. A. Ostanin, V. A. Kozlov

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 2, pp. 109-114, April, 2007
Original article submitted February 1, 2006

We studied the safety and efficiency of transplantation of autologous bone marrow cells in complex therapy of patients with spinal cord injury in the late period of the disease. In control group patients, meningomyeloradiculolis was performed, while in the main group surgical treatment was supplemented by transplantation of autologous bone marrow cells. Transplantation of BM stem cells into the cyst cavity and intravenously was well tolerated, did not cause allergic or inflammatory reactions in the early and delayed periods after surgery, and did not induce the formation of ossification foci in the nervous tissue. Analysis of the neurological status by ASIA, Bartel, and Ashworth scales showed that in the main group the positive clinical dynamics was more often observed than in the control. The decrease in neurological deficit included improvement of sensory and motor activity and conducting sensory function. Thus, transplantation of autologous bone marrow cells can be a novel safe strategy for the treatment of patients in the late period after spinal trauma.

Key Words: *spinal cord injury; stem cells; bone marrow; transplantation*

Traumatic injury to the spinal cord is an actual medical and social problem, because it often leads to severe disability of patients and requires considerable costs for their treatment and rehabilitation. The existing methods for the treatment of patients with complicated vertebrospinal traumas remain little effective, which leads to increasing number of patients with sustained neurological complications at the late periods of spinal cord injury (SCI). Surgical intervention in this period leads to recovery of the distal portion of the spinal cord in only 4% patients [1,4]. The use of modern drugs (nootropics, cholinoceptor agonists, corticosteroids, *etc.*) and electrostimulation does not cardinally change the situation [9]. This necessitates the search for principally new approaches to the therapy of SCI. Certain hopes in this field are related to cell therapy based on the use of stem cells (SC) [2,12].

Somatic SC present in practically all organs and tissues can undergo tissue-specific differentiation, are characterized by plasticity and migration activity, and, therefore, represent a considerable reparative reserve in the postnatal period [10]. The use of SC for stimulation of the repair processes in various pathologies is a new perspective trend called regeneration medicine. Identification of neuronal SC [14] and detection of neuronal differentiation of somatic SC from the bone marrow [8] prompted the use of SC for the treatment of SCI [13]. Indeed, significant decrease in neurological deficiency was observed after transplantation of

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences; Institute of Traumatology and Orthopedy, Novosibirsk. ***Address for correspondence:*** ct_lab @ mail.ru. E. P. Chernykh

0007-4888/07/1434-0543

bone marrow cells, in particular, mesenchymal SC, to experimental animals with modeled stroke, spinal trauma, and brain injury [5-7].

We performed controlled clinical trials for the evaluation of the safety and efficiency of autologous bone marrow cell transplantation (auto-BMT) in SCI patients at late terms of the disease.

MATERIALS AND METHODS

Treatment of SCI patients using auto-BMT was performed in accordance with Author's Patent "Method of Treatment of Spinal Cord Injury" (No. 2286160, priority from March 21, 2005). The study protocol was approved by Academic Council and Local Ethical Committee. The study enrolled 36 patients (26 men and 10 women) in late period of SCI, observed and operated in Neurosurgical Clinic, Institute of Traumatology and Orthopedy, in 2003-2006.

Inclusion criteria were the presence of cystic and/or atrophic degeneration and the absence of positive dynamics during the last 6 months. Patients of the control group (n=18) underwent meningomyeloradiculolis surgery; in the main group (n=18) surgical treatment was supplemented by auto-BMT. The groups were similar by patient's age, sex, SCI duration, localization, and character of damage (Table 1). Analysis of neurological status by ASIA, Bartel, and Ashworth scales (Table 2) also revealed similarity of the compared groups.

The procedure of trepanobiopsy and bone marrow aspiration were performed one day before surgery. Isolated bone marrow mononuclears were incubated for 24 h in DMEM supplemented with embryonic serum (10%) and the fractions of adherent and non-adherent cells were collected, washed, and resuspended in physiological saline containing 10% autologous plasma. The first fraction of cells was injected into the cystic cavity during surgery (the puncture hole was sealed with TachoComb), the second fraction was infused intravenously with a dropper.

Characterization of bone marrow cells included identification of hemopoietic SC and stromal mesenchymal cells in the population of isolated mononuclear cells. The relative content of hemopoietic SC was evaluated by the number of $CD34^+CD38^-$ cells using dual color flowcytofluorometry (FACS Calibur, Becton Dickinson). The number of stromal mesenchymal cells was evaluated by the number of their precursors identified as fibroblast CFU (CFU-F) according to Stem Cell Technology Protocol (2002). The reference group comprised 15 age- and sex-matched healthy volunteers (bone marrow donors).

RESULTS

A stable clinical picture of absent spinal cord function below the damaged segment was observed in all patients before surgery. Magnetic resonance imaging (MRI) of the damaged area revealed a focus of cystic-atrophic or atrophic degeneration.

Analysis of bone marrow aspirate revealed no disturbances in SC compartment in patients with SCI, which agrees with previous data [3]. The yield of mononuclear cells was the same as in healthy volunteers (Table 3). The relative number of $CD34^+CD38^-$ cells in SCI patients moderately surpassed the content of hemopoietic SC in healthy donors. The content of CFU-F in patients and donors was similar. These findings suggest that the bone marrow of SCI patients is a valuable source of SC by the content of hemopoietic SC and stromal mesenchymal cells and can be used for auto-BMT.

Auto-BMT was well tolerated, induced no allergic and/or inflammatory reactions in the wound and in the central nervous system, and was not accompanied by impairment of neurological symptoms in the early postoperation period and during later terms.

Analysis of the efficiency of treatment (9.4±4.6 months after surgery) revealed positive dynamics in 12 of 18 patients in the main group (Table 4), which manifested in improved segmental sensitivity in patients with trauma of the cervical portion (9 patients), upper thoracic portion (1 patient) and lower thoracic portion (2 patients) of the spinal cord. In 4 of these patients we observed improvement of segmental motor function and conduction functions of the spinal cord (recovery of nociception, surface sensitivity, and proprioception). Moreover, two patients noted improvement in the control of urinary bladder function. In the control group, positive changes after meningomyeloradiculolis were noted in 5 of 18 cases; they primarily manifested in improvement of segmental sensitivity. It should be noted that improvement in segmental motor and sensory functions in both groups was observed during the first months after surgery. Conduction sensory function in patients of the main group improved later (after 4-6 months) and persisted until the end of observation (2 years after surgery).

Evaluation of the neurological status in main group patients with trauma of the cervical part of the spinal cord by the ASIA scale revealed a significant increase in total sensitivity and motor activity score (from 44.3±5.0 to 58.3±7.2 and from 22.2±3.5 to 31.0±3.2, respectively, p<0.05). No changes of this kind were observed in the control group.

TABLE 1. Characteristics of Patients in the Main and Control Groups

Parameter	Main group	Control group
Sex		
men	14	12
women	4	6
Age, years	31.6 (18-47)	33.2 (23-53)
Time after trauma, months	36.4±7.9	33.7±5.5
Level of trauma		
cervical	12	8
upper and lower thoracic	2	5
lower thoracic/lumbal intumescence	4	5
Character of damage		
intramedullary cyst	11	11
subarachnoidal cyst	4	2
scar-adhesion process	3	5

Improvement of sensory function score (from 53.0±7.0 to 58.3±7.2) and motor function score (from 19.60±0.84 to 21.7±0.8) was minor and insignificant. In patients with other levels of SCI, no significant changes in sensory and motor functions were observed due to low number of observations. The decrease in neurological deficit affected social adaptation of patients. For instance, improvement by the Bartel scale in patients with trauma of the cervical part of the spinal cord was more pronounced in the main group than in the control (by 14.1±2.6 and 5.60±0.63, respectively, $p<0.05$). In patients with trauma of the thoracic and lumbal parts of the spinal cord, the more pronounced changes in the main group manifested as a clear-cut tendency. Evaluation of the dynamics of spastic syndrome (Ashworth scale) revealed significant decrease in spasticity in patients with trauma in the cervical part of the spinal cord in the main group: from 3.00±0.12 to 2.37±0.16 ($p<0.05$). At the same time in the control group, the decrease in spastic syndrome was less pronounced and insignificant (from 3.10±0.12 to 2.60±0.18; $p>0.05$).

MRI revealed no negative dynamics in the zone of SCI in the main group. In 3 patients with SCI in the cervical segment, the volume of intramedullary cyst and degeneration focus decreased 12 months after surgery. No ossification foci were found in the damaged zone. In the control group, aggravation of cystic and degenerative changes in the damaged zone was found in 2 patients during repeated examination.

The following case report can be a clinical example.

Patient L, 24-year-old female, admitted with a diagnosis "traumatic disease of the cervical part of the spinal cord, late period, and cystic degeneration of C_{IV}-C_V segments. Artificial bone-metallic block at the level of C_{IV}-C_{VI}, state after anterior decompression, anterior spondylodesis of C_{IV}-C_{VI} vertebrae. Distal paraparesis of the lower extremities, spastic paraplegia of the lower extremities. Disturbances of pelvic functions by the central type."

The patient complained the absence of active movements and elevated tone in her legs, weakness in arms, numbness of the body below the nipples. As seen from the history, the patients admitted Vladivostok regional hospital on July 29, 2003 after motor-vehicle accident with clinical picture of complete transverse injury to the spinal cord at the

TABLE 2. Parameters of Neurological Status of Patients in the Main and Control Groups (*M*±*SE*)

Group, level of trauma	ASIA		Bartel score, quality of life	Ashworth score, spastic syndrome
	motor activity, score	sensory function, score		
Control				
cervical (*n*=8)	19.60±0.84	53±7	19.4±2.4	3.12±0.10
upper and lower thoracic (*n*=5)	44.5±5.4	111±22	26±1	2.8±0.2
lower thoracic/lumbal intumescence (*n*=5)	56±6	155±14	29.0±1.9	1.4±0.7
Main				
cervical (*n*=12)	22.2±3.5	46.0±5.5	19.2±3.7	3.0±0.1
upper and lower thoracic (*n*=2)	50	112	27.5±2.5	2.5±0.5
lower thoracic/lumbal intumescence (*n*=4)	50	156.0±5.4	25±2	0.75

Note. *n*: number of patients.

TABLE 3. Characteristics of Bone Marrow Mononuclear Cells from SCI Patients and Healthy Donors

Parameter	Donors (*n*=15)	Patients (*n*=18)
Yield of mononuclear cells, 10^6/ml	7.6±1.8	10.9±1.0
$CD34^+CD38^-$, %	0.7±0.2	1.2±0.2*
CFU-F, $\times 10^6$ mononuclear cells	27.6±7.2	36.3±11.3

Note. *p<0.05 compared to donors (Wilcoxon—Mann—Whitney test).

cervical level, flaccid paralysis of the upper extremities and paraplegia of the lower extremities. Twelve hours after admission the patient underwent anterior decompression of the spinal cord and anterior spondylodesis of C_{IV}-C_V vertebrae with a Nickelide-Titanium implant. After surgery the patient noted minor recovery of the sensory function in her arms and partial recovery of shoulder movements. No significant positive dynamics was observed despite regular courses of rehabilitation treatment; therefore, the patients applied to Novosibirsk Research Institute of Traumatology and Orthopedy for further treatment.

At admission the patient was conscious, oriented, adequate, and critical. Low tone of the arms and hypotrophy of the hand and forearm muscles were noted. The strength of forearm and wrist flexors scored 4 and 3, the strength of forearm extensors scored 2, and the strength of hand muscles was 0. The total score of motor activity by the ASIA scale was 16. Spastic plegia of leg muscles with sharply increased muscular tone was noted. Anesthesia by the conduction type below C_{III}-C_{IV} segment involving the anogenital zone was observed. The sensory function by the ASIA scale scored 48. Spasticity by the Ashworth scale was up to 3. Adaptation score by the Bartel scale was 15. X-raying of the cervical spine revealed anterior bone-metallic block at the level of C_{IV}-C_{VI} vertebrae with 2.4% residual narrowing. MRI of the cervical part of the spinal cord revealed posttraumatic cystic and degenerative myelopathy with the formation of an intramedullary cyst at the level of C_{IV}-C_{VI} vertebrae (volume 1.7 cm^3).

In November 2004, meningomyeloradiculolis surgery and auto-BMT were performed: right-sided C_V hemilaminectomy, revision, emptying of the subarachnoidal cyst, and myeloradiculolis. No complications were noted in the postoperation period, the sutures were removed on day 12. The wound healed by primary intention. After 2 months, neurological deficit decreased: upper extremity strength and sensitivity increased by 8 (to 24 according to ASIA scale) and 16 (to 64) points, respectively. By the end of the first year after surgery, motor activity score attained 30 and sensitivity score attained 80. Adaptation score also increased (to 35, Bartel scale). The patient controlled functions of the urinary bladder and was able to stay on her feet with the help of a block and a support for up to 10 min; controlled spastics in the legs was achieved.

Medical aid to patients with traumatic SCI, especially in the late periods of the disease, is a serious problem all over the world, because of the absence of effective methods for the treatment of this pathology. Recently, the attention of neurosurgeons working in this field was drawn to the methods of cell therapy aimed at stimulation of regeneration and/or replacement of cell elements of the nervous tissue. Transplantation of SC is one of these methods [12]. The use of SC for the treatment of SCI was substantiated experimentally and approved in clinical practice. For instance, I phase clinical trials were performed evaluating the efficiency of intravenous or intraarterial (into vertebral arteries) injection of autologous bone marrow cells to

TABLE 4. Dynamics of Parameters of Neurological Status in the Examined Groups (*n*=18)

Character of changes, parameter	Control group		Main group	
	abs.	%	abs.	%
General positive dynamics	5	27.8	12*	66.7
Improvement				
segmental sensitivity	5	27.8	12*	66.7
segmental motor activity	1	5.6	4	22.2
conduction sensory functions	1	5.6	4	22.2
control over pelvic organ function	1	5.6	2	11.1
Deterioration	2	11.1	0	0

Note. *p=0.02 significance of differences in the frequency (Fisher exact test).

SCI patients in the subacute (n=8) and late (n=12) periods of the disease. The safety of bone marrow cell transplantation and clinical improvement (increased ASIA score) were demonstrated after intraarterial injection in 4 patients in the subacute period and in one of two patients in the late period and after intravenous administration in one of 4 patients in the subacute period [15]. In a clinical trial performed in South Korea, patients with a history of SCI up to 14 days received injections of bone marrow mononuclear cells along the perimeter of the damaged area in combination with administration of granulocyte-macrophage SCF. Transplantation of cells did not deteriorate the neurological status. Improvement of sensory and motor functions (ASIA score) was noted in 4 of 6 patients [11].

In out study we evaluated the efficiency of combined administration of bone marrow cells (into the cyst cavity and intravenous) in SCI patients at late terms of the disease. The absence of deterioration of neurological status and allergic/inflammatory side reaction in the postoperation period agrees with the data on sufficient tolerance of cell therapy. Moreover, repeated MRI of the damaged area revealed no pathological foreign tissue. This suggest that precursors of stromal mesenchymal cells present in the fraction of adherent cells after injection into the spinal cord do not form heterotopic ossification and chondrogenesis foci.

Evaluation of clinical efficiency showed that auto-BMT in combination with surgical treatment improves neurological dynamics in the majority of SCI patients. The mechanisms underlying the clinical effects of auto-BMT in SCI patients are not quite clear. It can be hypothesized that bone marrow cells produce neurotrophic factors stimulating neuronal growth and remyelination, activating regional precursors, and protecting nerve cells located in the damaged area from apoptosis. The administered suspension of bone marrow cells can contain endothelial precursors participating in the stimulation of angiogenesis, which can also promote regeneration of the nervous tissue. The replacement effect associated with neuronal differentiation of SC cannot also be excluded [7,13]. Further clinical and experimental studies will help to create new approaches improving the efficiency of therapy of SCI patients.

REFERENCES

1. M. A. Kosichkin, L. P. Grishina, and D. M. Shapiro, *Medical and Social Examination and Rehabilitation* [in Russian], No. 1 (1999).
2. D. S. Stankov, P. I. Katunyan, M. E. Krasheninnikov, and N. A. Onishchenko, *Vestn. Transplantol. Iskusstven.Organ.,* No. 1, 44-52 (2003).
3. E. P. Chernykh, E. Ya. Shevela, O. Yu. Leplina, *et al., Kletochn. Tekhnol. Biol. Med*., No. 1, 8-11 (2006).
4. M. B. Bracken and T. R. Holford, *J. Neurosurg. Spine*, **96**, Suppl. 3, 259-266 (2002).
5. J. Chen, P. R. Sanberg, Y. Li, *et al., Stroke,* **32**, No. 11, 2682-2688 (2001).
6. M. Chopp, X. H. Zhang, Y. Li, *et al., Neuroreport*., **11**, 3001-3005 (2000).
7. S. Corti, F. Locatelli1, S. Strazzer, *et al., Cur. Gene Ther*., **3**, 247-272 (2003).
8. C. P. Hofstetter, E.J. Schwarz, D. Hess, *et al., Proc. Natl. Acad. Sci. USA,* **99**, No. 4, 2199-2204 (2002).
9. C. E. Hulsebosch, *Adv. Physiol. Educ.,* **26**, Nos. 1-4, 238-255 (2002).
10. M. Korbling and Z. Estrov, *N. Engl. J. Med.,* **349**, Nos. 6, 570-582 (2003).
11. H. C. Park, Y. S. Shims, Y. Ha, *et al., Tissue Eng.,* **11**, No. 5-6, 913-922 (2005).
12. P. J. Reier, *NeuroRx*., **1**, No. 4, 424-451 (2004).
13. S. S. Schultz, *Current Drug Targets.,* **6**, No. 1, 63-73 (2005).
14. C. N. Svendsen, M. A. Caldwell, and T. Ostenfeld, *Brain Pathol*., **9**, 499-513 (1999).
15. E. Sykova, P. Jendelova, L. Urdzýkova, *et al., Cell. Mol. Neurobiol*., **26**, No. 7-8, 1111-1127 (2006).