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PHASE 1 TRIAL OF AUTOLOGOUS BONE MARROW STEM CELL TRANSPLANTATION IN PATIENTS WITH SPINAL CORD INJURY

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ABSTRACT

Introduction: A total of 18 patients, with complete motor deficits and paraplegia caused by thoracic and lumbar spine trauma without muscle atrophy or psychiatric problems, were included into this study.

Materials and Methods: The bone marrow was aspirated from the anterior iliac crest under local anesthesia and the mononuclear fraction was isolated by density gradient method. At least 750 million mononuclear-enriched cells, suspended in 2 mL of saline, were infused intrathecally.

Results and Discussion: The study reports demonstrated improvement of motor and sensory functions of various degrees observed in 9 of the 18 (50%) cases after bone marrow stem cell transplantation. Measured by the American Spinal Injury Association (ASIA) scale, 7 (78%) out of the 9 patients observed an improvement by one grade, while two cases (22%) saw an improvement by two grades. However, there were no cases in which the condition was improved by three grades.

Conclusions: Analysis of subsequent treatment results indicated that the transplantation of mononuclear-enriched autologous BMSCs is a feasible and safe technique. However, successful application of the BMSCs in the clinical practice is associated with the necessity of executing more detailed examinations to evaluate the effect of BMSCs on the patients with spinal cord injury.

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INTRODUCTION

Spinal cord injury is a disorder, which often causes the severe disability, including: incomplete tetraplegia, incomplete paraplegia, complete paraplegia and complete tetraplegia. The estimated lifetime costs for treatment and health care of one patient could be as high as 4, 5 million US $. According to the National Spinal Cord Injury statistical center, annual incidence in the USA is 40 cases per million populations, including only persons, survived after accident; annually there are 12500 new cases (www.nscisc.uab.edu). There is no cure or even any effective treatment for such patients. The main surgical procedure is decompression of spinal cord along with a high dose of methylprednisolone (Bracken, 2002). Though early decompression could have a neuroprotective effect, less than 1% of patients showed complete neurologic recovery by hospital discharge.

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Many patients remain in the wheelchair. Pharmacological agents such as methylprednisolone (Bracken, 1990 and Bracken, 1990), naloxone, monosialotetrahexosy lganglioside (GM-1), thyrotropin-releasing hormone (TRH) were studied in the clinical trials and no medicine have demonstrated strong evidences of clinical benefits (Hawryluk, 2008). Autologous stem cells could help the regeneration of injured spinal cord. Bone marrow mesenchymal and hematopoietic stem cells have differentiation potential. There are findings that BMCs differentiate into mature neurons or glial cells under experimental conditions (Muñoz-Elías, 2003 and Sanchez-Ramos, 2000). It was demonstrated that mesenchymal stem cell are able to give the neuronal like cells ex vivo, which express the neural cell marker. Preclinical study has shown that such differentiated cells were able to improve or restore damaged spinal cord function. Replacement, dedifferentiation or simply, paracrine effects were suggested. These findings showed that use of BMCs has the therapeutic potential in patients with neurological diseases. Different cell types were

used in preclinical studies for SCI treatment. Neural stem cells (NSCs), mesenchymal stem cells (MSCs), embryonic stem (ES) cells, olfactory ensheathing cells (OECs), Schwann cells (SC) pluripotent stem (iPS) cell have provided that they have regenerative potential (Silva, 2014).

Bone marrow *Stem Cells (Hematopoietic and Mesenchymal)*

MSC are multipotent cells. They have low immunogenicity, possess anti-inflammatory and immunosuppresive effects (Fehlings, 2011). Bone morrow derived MSC are the most widely used stem cells for SCI experiments. They differentiate into neurons and glia cells (Brazelton, 2000). Some authors suggested that cell fusion and transdifferentiation are the main mechanisms (Castro, 2002; Wurmser, 2002 and 2002). In animal models BM MSC were injected into spinal cord injury area (Ohta, 2004), or intrathecally (Ohta *et al.*, 2004) with some effects (Deng, 2006). Also expreiments in nonhuman primates and pigs were successfully in the mean of improvement of locomotor function (Ankeny, 2004 and Geffner, 2008). The secretion of growth factors and anti-inflammatory cytokines have been proposed as main mechanism in cell transplantation (Sharp, 2010; Kerr, 2010; Kanno, 2014). Clinical trials showed safety, and feasibility of BM MSCs transplantation for SCI patients. No serious complications were reported. The most patients showed improvement of motor and sensory functions. Представленная работа показывает phase 1 trial of autologous bone marrow stem cell transplantation in patients with spinal cord injury.

MATERIALS AND METHODS

Patients

Starting from March of 2012 until December of 2014, the total of 18 patients with complete motor deficits and paraplegia caused by thoracic and lumbar spine trauma without muscle atrophy or psychiatric problems, were included into this study. All patients signed a written informed consent. The study protocol was confirmed according to ethical guidelines of the 1975 Declaration of Helsinki and was approved by innova medical center). After Ethics Committee approval, following criteria had to be met to consider patients eligible for the trial: age – 18 - 65 years, gender - both.

Inclusion Criteria: subjects with chronic spinal cord injury (>12 months post-initial spinal cord injury surgery) who have stable neurologic symptoms for at least 6 months; Subjects with current neurological status of ASIA score A; The location of neurological injury of the patient is between C5 and T11; The injured site of the spinal cord is within three vertebral levels; Subjects must be able to read, understand and complete the Visual Analogue Scale (VAS) ; Subjects who have voluntarily signed and dated an informed consent form prior to any study procedures.

Exclusion Criteria: anatomical transection of the spinal cord; spinal cord lesion by sharp objects; ongoing infections; terminal, neurodegenerative or primary hematological diseases; Osteopathies which might increase the risk spinal cord puncture; Coagulopathies; Severe hepatic, renal or heart failure; Pregnancy or lactation.

Bone marrow cell therapy: 50-100 mL of bone marrow was aspirated from the anterior iliac crest under local anesthesia and placed in sterile tubes containing heparin. The aspirates were diluted 1:2 with PBS. The mononuclear fraction was isolated by density gradient centrifugation at 400 g for 30 min at room temperature using Ficoll Paque Plus or Ficoll Paque Premium solution (GE Healthcare, USA). At least 100 million enriched mononuclear cells, suspended in 2 mL of saline, was infused intrathecally.

Flow cytometry and viability testing: 0,04 ml of the final cell product was subjected to trypan blue dye exclusion test and flow cytomeric analysis. Viability test was performed by 0,4% trypan blue solution (Sigma, USA) according standard protocol. For cell immunophenotyping cell suspensions were incubated with anti-human CD34, anti-human - CD45, anti-human-CD271 (all from Miltenyi Biotec, Germany) and anti-human-STRO-1 antibodies (Santa-Cruz Biotechnology, USA) in 0,5% BSA/PBS (Sigma, USA) buffer according manufacturer instructions and flow cytometry analysis was carried out on BD FACSCalibur flow cytometer (Becton-Dickinson, USA). Mononuclear CD45-/CD34-/CD271+/STRO-1+ cells were defined as BM MSC and their percentage and absolute count were enumerated. Bone marrow hematopoietic stem cells were determined in CD45+/CD34+ mononuclear cell population and their percentage and absolute count were enumerated.

Follow-up Period: The follow-up visits were scheduled for 12 months after transplantation. During every follow-up visit, preoperatively and after 3 and 6 months from the surgery, the results were evaluated by assessing ASIA impairment scale (AIS), measuring electrophysiological parameters, including electroneuromyography and enhanced MRI.

Statistical analysis: Statistical analysis was performed by using SPSS Statistics v20 software. A paired sample t-test was used for determining whether or not there is a statistically significant difference between the results acquired before and after the treatment. Initial data (before treatment) was compared to that acquired at 6 and 12 months after treatment was performed. A significance level of 0,05 was chosen.

RESULTS

Transplantation of bone marrow stem cells was executed on 13 male (72%) and 5 female patients (28%), from 22 to 65 years of age. There were 12 (67%) patients with injury of the thoracic spine and 6 (33%) patients with lumbar spine. The period of time that has passed since the spinal cord injury (SCI) was from 12 to 20 months. According to the American Spinal Injury Association's classification of SCI AIS, there were 10 cases (56%) with AIS A score, 7 of which were males and 3 – females; 5 cases (28%) had B score, 4 of these were males and 1 – female; and there were 3 (17%) cases with AIS C score, out of which 2 were males and 1 was female. Out of 18 total patients, the reason of SCI in 11 (78%) cases was caused by car accidents, in 3 (17%) cases – by falling, and in 1 case – by a gunshot wound. The mean number of bone marrow cells (BMCs) infused in each patient was 776 million cells. All procedures were performed without any specific side effects or complications except for mild pain in the anterior iliac crest region at the sites of bone marrow puncture. Headaches were observed in 9% of the patients and temperature increased up to $37,5^0$C in 6% of patients, which lasted for two days. No other

complications or specific side effects related to the infusion procedure were reported. Continuous patient monitoring was carried out during the first 24 hours after transplantation. After this period patients were discharged. In this study, our attention was mainly focused on assessing the safety of this method. However, the AIS score and nerve conduction study reports have shown that after transplantation of bone marrow stem cells, out of 18 patients with SCI, the improvement of motor and sensory functions of various degrees was observed in 9 (50%) cases. As measured by AIS scale, in 7 (78%) out of these 9 patients, the improvement by one grade was observed and by two grades - in 2 (22%) cases. According to our preliminary researches, out of 18 total patients with SCI, the significant damage of the small and large tibial nerves does not occur on the electroneuromyography (ENMG) of 8 (44%) patients; in the form of insertional positive sharp waves and end plate potential, the bioelectric activity is registered in the lower limb muscles. After cell transplantation, in 5 (42%) of 12 patients suffering from urinary tract dysfunction, the improvements in urinary function of various degrees were observed. After cell transplantation, in 7 (78%) of 9 patients suffering from intestinal dysfunction, the improvement of bowel function in various degrees was observed.

DISCUSSION

Spinal cord injuries are accompanied by a number of complications, causing death of neurons, degeneration of nerve fibers, hemorrhage and eventually – the absence of complete regeneration in areas of injury. In most of the cases, traditional methods of treatment are very rarely able to restore the lost functions of tissues. However, the use of stem cells in such of patients gives hope for the opportunity to achieve functional and other improvements. Currently, human oligodendrocyte progenitor cells (OPCs), Schwann cells, bone marrow stromal cells, nasal olfactory ensheathing cells and others are being used for stem cell therapy during spinal cord injuries. Preclinical studies of the human OPCs application have shown that the effects of transplantation included robust white and gray matter sparing at the injury epicenter and, in particular, preservation of motor neurons that correlated with movement recovery (Sharp, 2010). One critical aspect of successful cell-based SCI therapy is the time of injection following injury (Kerr, 2010). The authors note that they injected the majority of transplanted OPCs at two clinically relevant times when most damage occurs to the surrounding tissues, 3 and 24 hours following injury. The derived OPCs expressed oligodendrocyte markers, including 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPa), galactocerebroside (GalC), oligodendrocyte transcription factor (Olig1), oligodendrocyte marker (O4, and O1). Moreover, OPCs survived when injected at the center of injury and migrated away from the injection sites after one week. Other authors think that human embryonic stem cell-derived OPCs can be transplanted sooner than conventionally accepted.

Transplantation of Schwann cells can also be a promising therapeutic strategy for spinal cord repair. They are one of the most widely studied cell types for repairing the spinal cord. Unlike oligodendrocytes and their precursors, Schwann cells possess many of the characteristics that are desirable for transplantation in spinal cord lesions. They can be easily collected from peripheral nerve, easily purified and grown in culture in large quantities. Due to their ability to dedifferentiate, migrate, proliferate, express growth promoting factors, and myelinate regenerating axons, Schwann cells play a crucial role in endogenous repair of peripheral nerves (Kanno, 2014). The transplantation of Schwann cells in the injured spinal cord boosts the regeneration of axons, myelinates or ensheathes regenerated axons in a normal way, reduces cyst formation in the injured tissue and reduces secondary damage of the tissues around the initial injury site [22]. In order to improve the clinical condition of the patients with SCI, the cultured and purified autologous Schwann cells, that were previously isolated from the sural nerve, were transplanted [23]. As it is noted by authors, there were some signs of improvement in the autonomic, motor and sensory function of all patients. Authors report that they have assessed the safety and feasibility of a combination of bone marrow mesenchymal stromal cells and Schwann cells for the treatment of patients with chronic spinal cord injury [24]. However, when transplanting Schwann cells, the regeneration and myelination occurs only where the graft is located. Due to the inhibitory nature of the glial scar surrounding the injury axons do not regenerate beyond the graft [25]. Thus, despite that the Schwann cells have great potential for repairing the injured spinal cord, in order to successfully use them, the researches continue to address issues such as encouraging the survival and growth of damaged axons using neurotrophins, which can help with the establishment of appropriate connections between regenerating axons and target neurons and thus, provide functional recovery [26], by neutralizing inhibitory molecules associated with the failure of axonal regeneration [27] and many others. Another promising candidate for cell transplantation in SCI cases may be the olfactory ensheathing cell.

This is due to the unique capabilities of these cells as they are being continually replaced throughout the life, and also to the fact that the rate of neurogenesis can be regulated by manipulating the system in order to abbreviate or prolong the average life of a sensory neuron [28]. Phase 1 clinical trial, conducted in a single blind test, has shown that, up to one year post-implantation, the transplantation of autologous olfactory ensheathing cells into the injured spinal cord is feasible and safe [29]. However, there are contradictory opinions about the effectiveness of transplantation of olfactory ensheathing cells for spinal cord injury. Thus, some authors report that together with Schwann cells and olfactory ensheathing cells created a 3-dimensional matrix that provided a permissive microenvironment for successful axon regeneration in the adult mammalian central nervous system 30]. Others report that transplantation of Schwann cells or olfactory ensheathing glia, or their combination in the adult Fischer rat thoracic (T9) spinal cord, after 1 week from a moderate contusion, Schwann cell graft is more effective in promoting axonal sparing/regeneration rather than the combination of Schwann cells with olfactory ensheathing glia or olfactory ensheathing glia graft [31].

Special interest is paid to bone marrow hematopoietic and mesenchymal cells. South Korean group has reported 35 patients with spinal cord injury, treated with autologous bone morrow stem cell transplantation into the surrounding area of injury along with GM-CSF injection. In a phase I/II open-label nonrandomized study patients were transplanted with BMCs in acute (within 14 days after injury, subacute (2 -8 weeks) and chronic patients (more than 8 weeks). Control group patients were treated with conventional decompression and fusion surgery without BMC transplantation. At 4 months, the MRI

demonstrated the spinal cords enlargement without any hemorrhage, new cysts, or infections. The AIS grade increased up to 30-33 % of the acute and subacute treated patients, respectively, (AIS A to B or C), whereas no significant improvement was observed in the chronic treatment group [32]. In 2007 Sykova et al. published study on 20 complete spinal cord injury patients. Patients were transplanted 10 to 467 days postinjury. Intraarterial bone morrow cell (via catheterization of a. vertebralis) transplantation was successfully in acute patients group (10-30 days postinjury). Patients were evaluated with ASIA protocol and Frankel score, MRI Evaluation and electrophysiology: motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs). Motor and sensory function was improved in most patients within 3 months. No complications were observed [33]. In 2008 Geffner et al. has reported eight case of treatment of SCI (four acute, four chronic) with bone marrow stem cells. Cells were injected via multiple routes: directly into the spinal cord, intrathecally and intravenous. For neurological evaluation ASIA, Frankel, and Ashworth scales were used. Comprehensive evaluations demonstrated improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring. ASIA Motor Score/Sensory Light Touch Score/Sensory Pin Prick Score were improved in all 8 patients as well as Barthel Index Score and Bladder Function Score and showed stable improvement even after 2 years after treatment (Geffner, 2008). The similar results were obtained by authors that transplanted mesenchymal bone marrow stem cells in 40 patients with SCI.

The cells were transplanted in the area surrounding the injury. During the whole period of observation there were significant improvements in the patients that had no serious complications [34] Other authors report that they obtained satisfying results using the transplantation of autologous bone marrow-derived cells in addition with physical therapy in patients with chronic cervical and thoracic SCI; the duration of the injury in these patients was at least 12 months. The injection of stem cells was conducted with intrathecal injection [35]. The others obtained satisfying results when treating the patients with SCI using hematopoietic progenitor stem cell [36]. According to our preliminary researches, the prognosis for SCI patients may depend on various reasons, including the etiology of SCI, the time elapsed since the injury, the age of the patient, the type of stem cells that are more suitable for transplantation in patients with SCI, the amount of these cells, the ways to deliver the cells into the lesion and others. The further successful application of stem cell therapy in the patients with SCI depends largely on solving the above mentioned issues.

Conclusion

Despite the fact that we have not performed a statistical analysis, some patients showed clinical and electrophysiological evidence of improvement. It is significant that here were no significant complications of autologous MSC transplantation. However, successful application of the BMCs in the clinical practice is associated with the necessity of executing more detailed and deep examinations to evaluate the effect of BMCs on the patients with spinal cord injury.

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