Case Report

Evaluation of Somatosensory Evoked Potential and Pain Rating Index in a Patient with Spinal Cord Injury Accepted Cell Therapy

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Manuscript received: 05-19-2015 Accepted for publication: 12-17-2015

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Spinal cord injury (SCI) causes a high incidence of motor and sensory dysfunctions accompanied with neuropathic pain. No effective treatment is available. Both somatosensory evoked potential (SSEP) and neuropathic pain (NPP) are transmitted via myelinated large diameter fibers of deep sensory pathways. Here we aimed to evaluate whether SSEP can consistently and objectively assess transmission of deep sensory pathways, and to examine the effects of umbilical cord mesenchymal stem cell (UCMSC) transplantation on SSEP and NPP as assessed by the pain rating index (PRI) in a patient with a 2-year history of complete cervical SCI. We demonstrate that SSEP can directly reflect physiological function of myelinated large fibers in deep sensory pathway transmission (NPP is also transmitted by the same pathway). One year after UCMSC transplantation, the SSEP parameter, PRI, and clinical presentations of NPP significantly improved.

Key words: Spinal cord, neuropathic pain, somatosensory evoked potential, umbilical cord mesenchymal stem cells

Pain Physician 2016; 19:E659-E666

The global incidence of spinal cord injury (SCI) is approximately 15 – 73 per million people (1,2). The economic and social burden is on the rise. Currently, the treatment of SCI mainly focuses on rehabilitation; however, outcomes are unsatisfactory. Stem cells have been applied to treat traumatic injury of the nervous system such as cerebral palsy, brain trauma, and SCI for neural repair (3,4). Umbilical cord mesenchymal stem cells (UCMSC) are a common type of stem cell with the advantage of abundant resources, low immunogenicity, immune escape (5,6), and a strong ability for proliferation, making them suitable for clinical treatments.

SCI causes motor and sensory dysfunctions due to damaged neural pathways. Additionally, up to 90% of SCI patients present with various types of pain including neuropathic pain (NPP), which is defined as a direct consequence of a lesion or disease affecting the somatosensory system. The large diameter myelinated fibers (Aβ fibers) conducting somatosensory evoked potential (SSEP) contribute to the pathophysiological changes in NPP (7). SSEP mainly represents the centripetal conduction of the sensory pathway induced by stimulating any point on the sensory nerve conduction route, including receptors and peripheral nerves. To some extent, SSEP reflects the functions of somatic sensory afferent pathways, the brain stem, and cerebral cortex. It has been shown that SSEP is conducted by the large diameter myelinated fibers, which constitute the posterior column-medial lemniscus pathway. Therefore, severity of NPP caused by damaged myelinated fibers could be assessed by analyzing SSEP, which objectively reflects physiological functions of the remaining fibers.

Here we aimed to evaluate whether SSEP recordings and PRI assessment objectively reflect transmission of deep sensory pathways, as well as to examine the effects of UCMSC on SSEP and NPP. To this end, we assessed SSEP, the pain rating index (PRI), and clinical presentations of NPP prior to and after UCMSC transplantation in a patient with complete SCI.

Case Report

The patient was a 25-year-old man with a 2-year history of traumatic complete cervical SCI. His upper trunk was squeezed horizontally by a collapsed wall, which caused the fracture at the level of the sixth cervical vertebra. He received open reduction decompression and internal fixation on the twenty-seventh day after injury in the Department of Cell Therapy, General Hospital of Chinese People's Armed Police Forces; however, he remained paraplegic with urinary and fecal incontinence after the surgery. Loss of proprioception and superficial sensation below the level of costal angle was observed. During hospitalization and within one year after discharge, the patient did not take any neuro-nutrient medications and did not receive further surgery, and he continuously performed the same amount of exercise as before without systematic rehabilitation in the hospital.

Stem Cell Transplantation (SCT) Therapy

The cell therapy protocol was approved by the Ethics Committees of the General Hospital of Chinese People's Armed Police Forces. Prior to the treatment, a consent form was signed by the patient and his guardian confirmed their willingness to accept transplantation of UCMSC.

The method we used to obtain UCMSC was the same as described previously (4). Briefly, a healthy pregnant woman voluntarily donated her umbilical cord (UC). After successful childbirth, an approximately 10 cm umbilical cord containing Wharton's jelly was obtained. Wharton's jelly was then separated, rinsed, cut into small pieces of 0.5 cm^3, centrifuged and routinely cultured in Dulbecco's Modified Eagle Medium (Thermo Scientific, USA) containing 10% fetal bovine serum (Biochrom AG. Leonorenstr, Germany). The residual UC tissue blocks were removed from the medium after 10 days, while cells attached to the dish wall continued to be cultured until the rate of cell fusion reached more than 80%. The cells were trypsinized with 0.25% trypsin (Thermo Scientific, USA) and passaged every week. The third or fourth generation of cells was selected for treatment after characterizing mesenchymal stem cell markers by flow cytometry.

ECG, chest x-ray, full blood examination, and biochemistry were performed to exclude infection and cardiac pulmonary dysfunctions within 3 days after admission. On the fourth day, 2 mL of UCMSC was suspended in normal saline at a concentration of 5×10^6/mL was transplanted into the cerebrospinal fluid by intrathecal injection 4 times over the span of 3 days.

Methods and Results of Flow Cytometric Analysis of UCMSC

Flow cytometry (FACSCalibur; BD, New Jersey, USA) was used to identify UCMSC, and immunofluorescence-labeled monoclonal antibodies and reagents were obtained from BD Company (New Jersey, USA). CellQuest Pro software was used for analysis. The results showed that these cells expressed CD90 (99.98%), CD44 (98.53%), CD105 (99.56%), CD73 (99.97%), CD45 (0.42%), CD19 (0.64%), CD11b (0.20%), and CD34 (0.55%) (Fig. 1).

SSEP Stimulation and Recording

Medelec Synergy 11.0 evoked-potential instruments (Oxford Instruments, England) were used for potential recording.

SSEP Recording of the Posterior Tibial Nerve

A bipolar electrode was used to stimulate the posterior tibial nerve at 2 – 3 cm posterior to the bilateral medial malleolus. The anode was placed near to the proximal end (the constant current stimuli were rectangular electrical pulses: wave length, 0.1 ms; frequency, 3 pps; intensity, 20 – 35 mA; foot plantar flexion indicated successful stimulation). The electrode on the upper limb was connected to ground, an electrode was placed at Cz (i.e., the recording was performed at 2 cm posterior to Cz on the midline), and FPz was used as a reference (EEG International 10 – 20 system). Electrode impedances between each electrode and the scalp were less than 5KΩ. Electrodes for recording were placed at the ipsilateral popliteal and the spinous process of T12; electrodes next to the knee and at the ilium were used as reference points.

SSEP Recording of the Median Nerve

A bipolar electrode was placed at 2 – 3 cm near the distal end of the wrist wrinkle to stimulate the median nerve. An anode was placed near the proximal end (the constant current stimuli were rectangular electrical

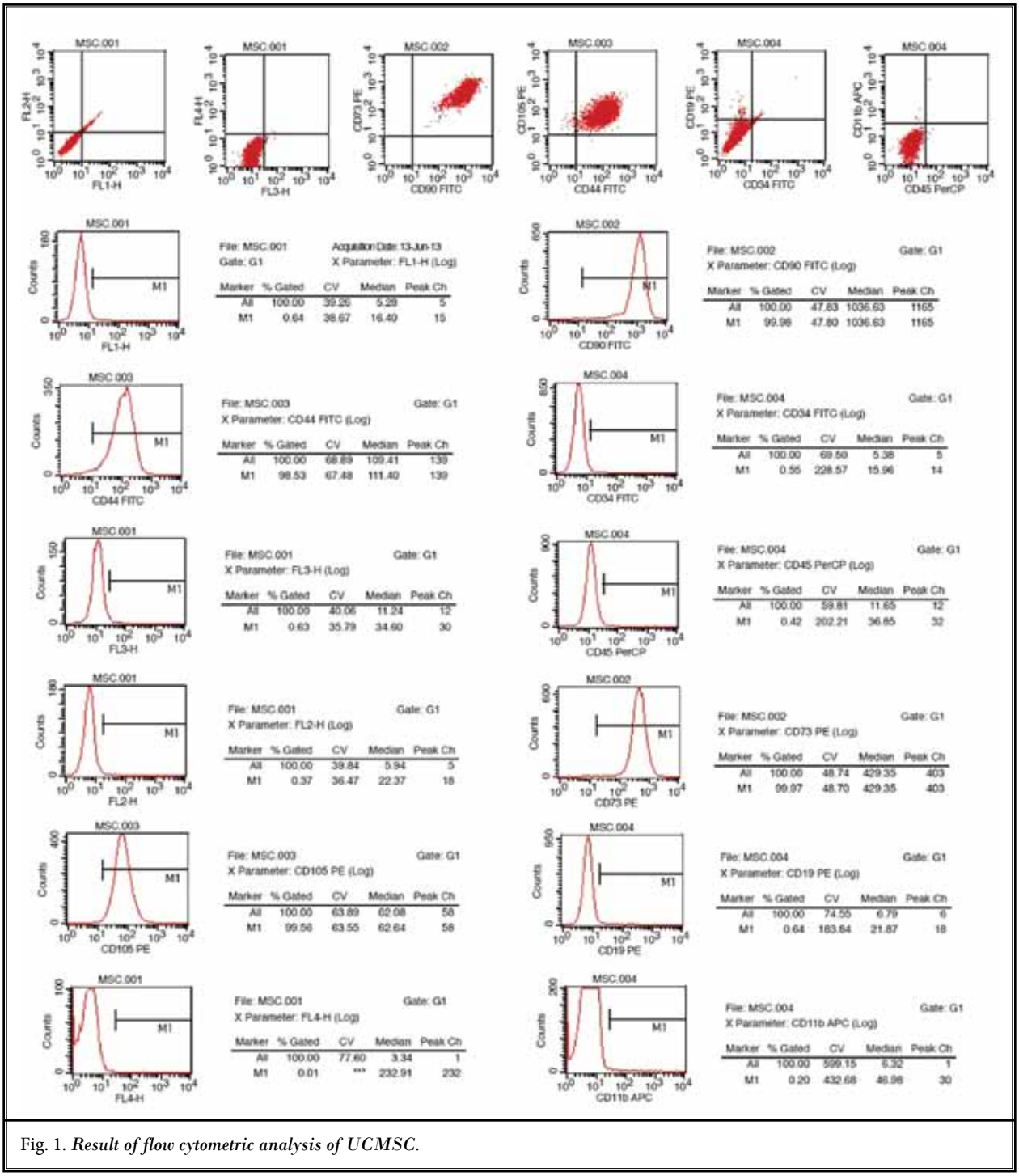

Fig. 1. *Result of flow cytometric analysis of UCMSC.*

pulses: wave length, 0.1 ms; frequency, 3 pps; intensity 10 – 25 mA; finger dorsal flexion indicated successful stimulation). The electrode on the upper limb was connected to ground. FPz was used as a reference. Electrode impedances between each electrode and the scalp were less than 5KΩ. Electrodes for recording were placed at the ipsilateral supraclavicular fossa (Erb') and 1 – 2 cm above the spinous process of C7 and C4' (for

recording of SSEP on the left median nerve) or C3′ (for recording of SSEP on the right median nerve).

For recording in the posterior tibial nerve or in the median nerve, the range of bandpass was chosen as 3 to 3000Hz, analysis time was 100 ms, and sensitivity was 5 μV. Recordings were made separately on the left and right sides. Two hundred potentials on each body side were recorded and superimposed. Each value was measured in triplicate, and each electrophysiological parameter was acquired from stable waves to ensure reproducibility.

Results of SSEP Recordings

Two years after SCI, the patient received first SSEP recordings prior to SCT. Based on the Classification of American Spinal Injury Association (ASIA), ASIA sensory scores were 8 on both left and right sides, while ASIA motor scores were 6 on the left side and 2 on the right side. ASIA was classified as A. Results of SSEP recordings in the upper limbs showed that N9 latency was normal, and no defined wave was produced after repetitive trials on bilateral C7 and C3′ (C4′). Results in the lower limbs showed that N6 latency was normal and that no defined waves were identified on the bilateral T12 and Cz (Fig. 2).

One year after SCT (i.e., 3 years after injury), second SSEP recordings were performed. ASIA was again classified as A. The ASIA sensory score remained 8 on both sides, while ASIA motor scores were 11 on the left side and 6 on the right side. Results of SSEP recordings

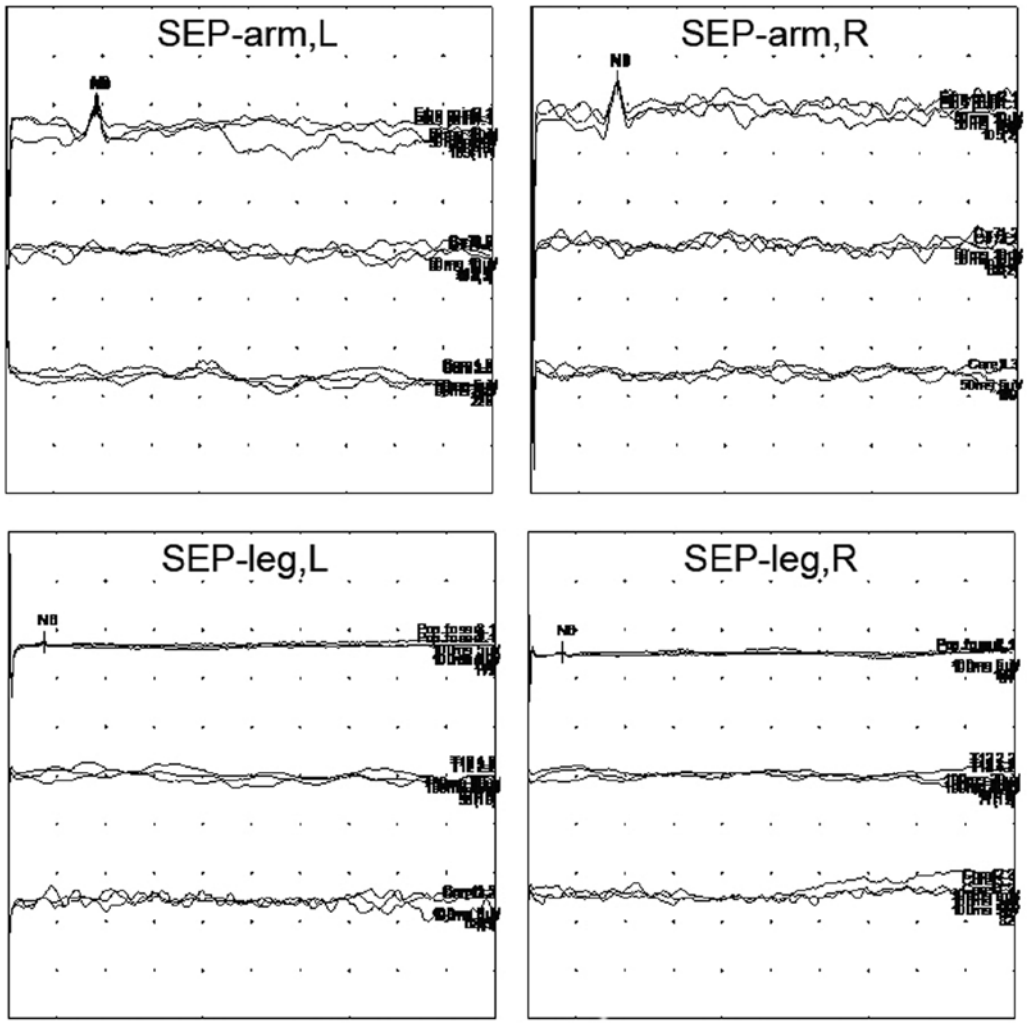

Fig. 2. *SEP results before SCT therapy*.

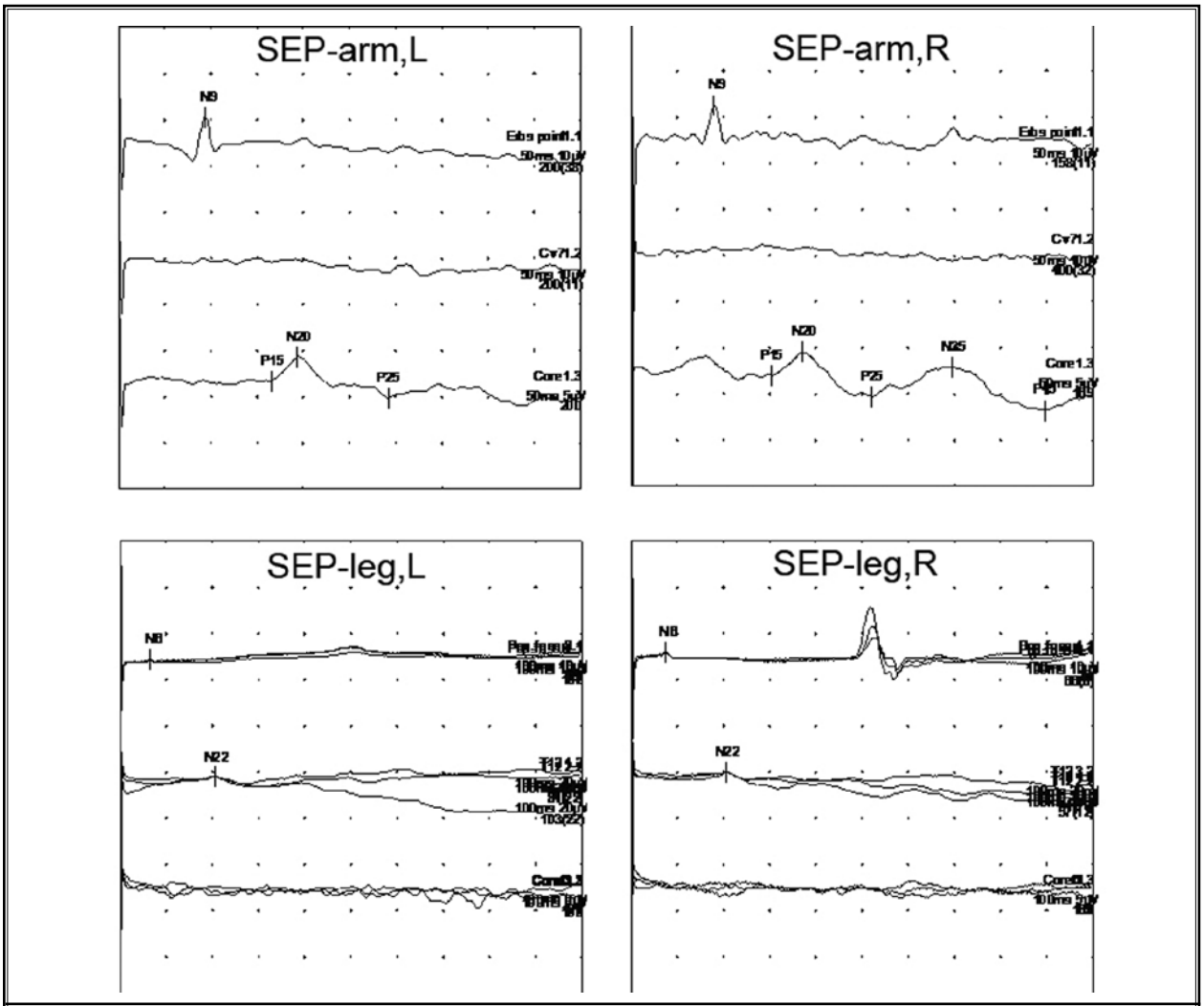

Fig. 3. *SEP results after SCT therapy.*

in the upper limbs showed that N9, P15, and N20 latencies were normal; P25 component eventually appeared but with a prolonged latency. No defined waves were identified on the bilateral C7 after repetitive trials. Recording in the lower limbs showed that N6 and N22 latencies were normal, and that no defined waves were identified on Cz after repetitive trails (Fig. 3).

Assessment of PRI

The McGill Pain Questionnaire (MPQ) is an international standard scale for description and evaluation of pain. The Short-form McGill Pain Questionnaire (SF-MPQ) is simplified on the basis of MPQ and has high reliability in clinical applications. Prior to transplantation, the score of PRI was 8 in the sensory category, 11 in the affective category, with the scale totaling 19. The most obvious pain experienced by the patient was shooting pain. One year after the transplantation, the score was 3 in the sensory category, 0 in the affective category, and the total of scores was 3. The total of pain scores was decreased by 16 after transplantation (Fig. 4).

Clinical Presentations

Prior to SCT, proprioception, vibration, and 2-point discrimination below the costal angle all disappeared. One year after SCT, the patient felt strong palpation on the abdomen, though proprioception and vibration on the feet did not improve. Moreover, both pain and

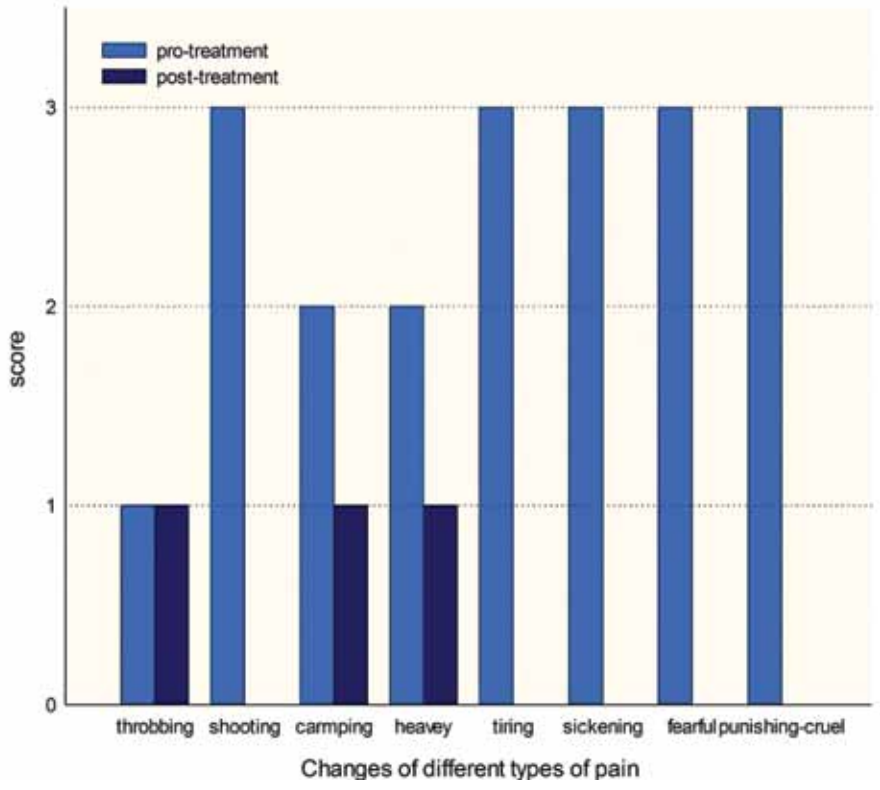

Fig. 4. *The score of different types of pain pro-tretment vs. post-treatment.*

temperature sensation were persistently absent below the costal angle both prior to and after SCT.

Discussion

Poor neural regeneration severely affects functional recovery of patients with SCI, particularly complete transverse SCI. Stem cell therapy provides new hope for SCI treatment as it has been shown that (1) these cells can be differentiated into various cell types under certain conditions; (2) that they secrete many cytokines to provide nutrition and repair damaged cells; (3) they can improve cell survival; (4) they can promote angiogenesis; and (5) they have been shown to inhibit scar formation (8-12).

The spinal cord receives signals from peripheral sensory receptors and conducts nerve impulses up to the brain. Afferents in the sensory pathways can be easily damaged and are very difficult to recover, which is the main cause of sensory dysfunctions after SCI. SCI induces an increase in the expression of sodium channels and voltage-gated calcium channels in cell membranes, and enhances the release of inflammatory factors as well as increases the number of sympathic nerve fibers. Moreover, following SCI, myelinated low-threshold Aβ fibers replace high-threshold C-fibers to form new synapses. Consequently, SCI leads to activation of neurons which normally do not response to C-fibers, leading to central sensitization that increases neural excitation and induces abnormal response to pain (13,14). Other studies have shown that myelinated Aβ fibers are the pathological basis of NPP (7), and it is known that SSEP is transmitted by these fibers in the posterior column-medial leminiscus pathway. Therefore, SSEP as an objective and indicative electrophysiological recording is usually applied to measure the functional integrity of spinal somatosensory pathways. Here we reported a marked reduction in pain scores and a series of changes in SSEP in a patient with complete SCI after SCT. Before receiving of SCT, the N9 component was present but P15 and N20 components were absent, indicating that

the medial lemniscus pathway was damaged and that deep sensory dysfunctions from the spinal cord to the cortex caused the absence of postsynaptic and cortical potentials.

After SCT, P15 and N20 components appeared and latency was normal while the P25 component was present with long latency and N13 was always absent, suggesting that the posterior spinocerebellar tract-medial lemniscus did not generate postsynaptic potential, and that the presence of N20 and P25 components indirectly demonstrated abnormality of SSEP afferents, that is, the generation of cortical potential does not solely depend on myelinated large fibers in the medial lemniscus, transmission of the potential could occur in other fibers as well. It is possible that axons and/or dendrites are formed from new neurons differentiated after SCT or from already existing neurons, and that they transmit nerve impulses laterally. SSEP recordings in the anterior lower limbs showed the presence of N6 component and normal latency in the absence of other waves, indicating that dysfunction of sensory transmission occurs from the spinal cord to the cortex. This result was consistent with the results of SEEP recording in the upper limb. After SCT, the N22 component was present and its latency was normal, N22 being a postsynaptic potential generated in the grey matter of the spinal cord and representing the response to axonal input from lateral branches and the existence of axonal transmission of these branches; however, cortical potentials of P40 and N55 components were absent even after SCT, suggesting even though lateral branches of axons of neurons form along pathways, no sensory transmission occurs.

The difference in the cortical potentials of SSEP between the median nerve and posterior tibial nerve may be due to anatomic structures. Processes of first-order neurons in the deep sensory pathways constitute the posterior column. Afferents below the T5 ascend forming the gracile fasciculus in the inner portion of the posterior column. Afferents above the T4 ascend forming the cuneate fasciculus. These fasciculi ascend parallel to the medulla oblongata to form the gracile nucleus and cuneate nucleus. Vascular supply of the spinal cord is mainly from branches of the intercostal arteries on the surface of the spinal cord, and therefore the cuneate fasciculus can receive a relative abundance of blood supply which is beneficial to nerve regeneration after injury in the upper trunk.

Conclusion

In the present study we showed that UCMSC transplantation can partially promote recovery of deep sensory pathways after SCI as demonstrated by SSEP recording and alleviated NPP. Thus, SSEP recording can directly and effectively reflect physiological function of myelinated large fibers in the deep sensory pathway, and the improvement in transmission of these pathways correlates with the alleviation of neuropathic pain.

Conflict of Interest

The authors declare no conflicts of interest in regard to the present manuscript.

Acknowledgments

This study was supported by the General Hospital of Chinese People's Armed Police Forces Hospital Fund (WZ20130306) and the Department of Science and Technology, Guangdong Province, China (No. 2011A081401003).

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