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Human Embryonic Stem Cells in the Treatment of Spinocerebellar Ataxia: A Case Series

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Abstract

Spinocerebellar ataxias, dominantly inherited ataxias, constitute a large heterogenous group of progressive neurodegenerative disorders that commonly affects the cerebellum and its afferent and efferent pathways. No pharmacological treatment has been found to be effective in the treatment of spinocerebellar ataxias. Stem cell-based therapy is emerging as a promising therapeutic option for the treatment of Spinocerebellar ataxias. In this case report, three patients with spinocerebellar ataxias were treated with human embryonic stem cells. Following the treatment, all patient showed noticeable changes in their health such improvement in hand eye coordination, gait pattern, ability to stand without support, muscle strength in all the limbs, ability to walk and turn while standing without support, clearance in speech, good energy levels, reduction in twitching of cheek muscle, stamina, endurance and coordination.

Keywords: Spinocerebellar ataxias; Human embryonic stem cell therapy; Stem cell therapy

Abbrevations: SCA: Spino Cerebellar Ataxias; hESC: Human Embryonic Stem Cells; HuUCBMCs: Human Umbilical Cord Blood Stem Cells; MSCs: Mesenchymal Stem Cells

Introduction

Spino Cerebellar Ataxias (SCA), dominantly inherited ataxias, constitute a large heterogenous group of progressive neurodegenerative disorders that commonly affects the cerebellum and its afferent and efferent pathways [1,2].

Demise of neurons in SCA is known to be caused by mutant proteins that interfere with several molecular pathways including transcriptional regulation, protein aggregation, alterations of calcium homeostasis, activation of pro-apoptotic routes among others and the ubiquitin-proteasome system. This states the need of therapeutic options that could simultaneously target multiple pathways [3].

A review on the use of pharmacological treatments including physostigmine, choline and its derivatives, tyrotrophin releasing hormone, serotonin, sulfhamethoxazole-trimethoprim, acetazolamide and cycloserine showed that no study till date have confirmed any of these treatment to be an effective option for cerebellar ataxia [4].

Stem cell-based therapy is emerging as a promising therapeutic option for the treatment of SCA. Studies on the use of human umbilical cord blood stem cells (HuUCBMCs), neural stem cells, induced pluripotent stem cells, human embryonic stem cells (hESCs), mesenchymal stem cells (MSCs) and fetal cells in the treatment of cerebellum-related disorders have showed them to possess neutroprotective potential [5-9]. In our previous studies we have shown improvement in the patient's condition who were suffering from cerebral palsy and cortical visual impairment after hESCs therapy (papers accepted in press). Our present study reports the use of hESCs in the treatment of patients with spinocerebellar ataxia. All these patients had come to our facility after they did not benefit from their previous traditional therapies. We started hESC therapy as a primary treatment for all the patients and a standard protocol for administration of hESCs was followed.

Methods and Material

hESCs are cultured and maintained as per our proprietary in-house patented technology in a Good Manufacturing Practices, Good Laboratory Practices and Good Tissue Practices certified laboratory at Nutech Mediworld (Patent-WO 2007/141657A PCT/1B 2007 Published 13 Dec 2007). The evidence for the use of hESCs at Nutech Mediworld has been submitted in written and accepted at House of Lords, Regenerative Medicine, Science and Technology Committee [10] . The cell lines are free of animal product and are chromosomally stable.

Cells were administered through different routes intramuscular (i.m) route twice daily to "prime" the body and preparing the recipient immune system not to reject the stem cells, 1 ml hESCs (<16 million cells) were administered twice a week through intravenous (i.v) route to "home in" to the required area and 1 to 5 ml hESCs were administered every 7 days by any of the supplemental routes (epidural infusion or injection, subarachnoid injection, or deep spinal injection) to introduce the stem cells. These patients were also subjected to nasal sprays of hESC (twice a week) and deep spinal muscle injection (twice a week).

Treatment protocol consisted of different phases and a gap phase in between. T1 phase consisted of 12 week followed by subsequent phases T2 (4-6 weeks), T3 (4-6 weeks) with same dosage regimen as described above. It takes almost 14-16 weeks for a human fetus to develop complete organs [11]. So, the duration of gap phase between different treatment periods was decided keeping into mind the time taken for organogenesis. All the patients received the therapy using the same treatment protocol. On an average, a patient receives hESC therapy for 24 weeks in a year (T1 to T4) and about 20-23 mL of hESCs are administered per week. Thus, in a year a patient receives approximately 480 mL of hESCs. One mL of cell suspension has about 4-5 million cells.

Informed consent was provided by all the patients prior to start of the treatment. The condition of patients was video graphed before,

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Cases	Age (Years)	Admission Date	Chief Complaints
1	65	30 Dec 03	Increased frequency of micturition since 5 years, tremors and difficulty in walking and swallowing
2	59	1 Feb 12	Difficulty in speech since last 28 years, difficulty in maintaining balance since last 25 years, difficulty in writing since last 14 years, difficulty in walking since 19 years, memory loss since 3 months and sleeplessness since 28 years
3	41	1 Feb 12	Tremors of the upper limbs (ULs), unsteadiness of gait, walking imbalance, slurred speech and difficulty in negotiating narrow passages

Table 1: Characteristics of Patients.

during and after the treatment periods. SPECT scan was done before the start of the treatment and then at regular intervals to document perfusion in the brain. Doctor and nurses at the facility were trained to observe the patients for antigenic or paraphylactic reactions following the administration of hESC therapy.

The SPECT scan (Millennium MG, GE) was carried out before or within 7 to 10 days of hESC therapy initiation and thereafter at the end of each treatment phase. Patients showing 10%-30% changes were considered to have mild improvement, 30%-60% changes to have moderate improvement and 60%-90% changes as significant improvement. Patient characteristics are presented in Table 1.

Case 1

A 65-year old female was admitted to Nutech Mediworld on 30 December 2003 with chief complaints of increased frequency of micturition since 5 years, tremors and difficulty in walking and swallowing. Patient's family history revealed that she had father, brother and three sisters (two twins) affected with the same disorder.

Patients history revealed that she was apparently well until the age of 38 years when she had several unexplained falls. Gradually there was a loss of balance with more falls and right leg dragging (Rt) more than left (Lt) leg. Patient complained of bilateral intentional tremors which interfered with eating and writing. She also noticed change in speech and tremors in her voice. Other symptoms included forgetfulness and personality changes. She also related sleeping difficulties and early awakenings.

The patient also complained of urine urgency and increased frequency of micturition. She required intermittent catheterization. She reported difficulty in walking and difficulty in swallowing (both liquids and solids). Other symptoms included back ache and tiredness.

The patient was a known case of SCA since 15 years. Patient had a past history of axillary neuropathy (Rt) after dislocation of shoulder and had undergone laser treatment for bilateral glaucoma. She had a strong family history with her father, brother and sister suffering from the same disorder.

On examination of Central Nervous System (CNS), the patient was unable to walk without support, had mild cognitive impairment. She had slow disarticulated speech with low tone and small shuffling gait with poor balance. Romberg sign and head nodding were positive. Deep tendon reflexes were exaggerated. Presence of signs including intentional tremors, head nodding, nystagmus and Romberg's positive and no coordination in finger nose tip test confirmed that the patient had cerebellar ataxia. The patient was also not able to speak well as she had difficulty in breathing. Her speech was present only during expiration. There were continuous tremors all over her body including neck and head. SPECT scan of the brain revealed hypoperfusion.

Patient was given hESC therapy as primary treatment along with extensive physiotherapy. Patient has taken hESC therapy on regular basis since 2003. There have been noticeable changes in the health of patient after treatment. The patient showed improvement in overall stamina, endurance, coordination, sitting balance, standing and walking ability, speech and flexibility. Reduction in tremors occurred and she was able to hold objects and tried to put them at specific place. With no head nodding, the patient was able to eat food on her own. She is alert and her bladder dysfunction is absent. SPECT scan revealed improvement in the patient. It showed decreased area of hypoperfusion. The patient's condition has not deteriorated and is stable. However, her twin sister who did not get the treatment died in 2009.

Case 2

A 59-year old male doctor was admitted at Nutech Mediworld on 1 February 2012 with chief complaints of difficulty in speech and sleep since last 28 years, difficulty in maintaining balance since last 25 years, difficulty in writing since last14 years, difficulty in walking since 19 years and memory loss since 3 months.

The patient was apparently well 28 years ago when he first started facing difficulty in speech and 3 years later, he reported impairment in balancing during walking. Gradually, his handwriting got worse. He suffered from severe depression after initiation of symptoms. He also reported insomnia since 28 years and memory loss since past 3 months. There was a twitching of cheek muscle. Other symptoms included back pain while exercising.

Patient was a known case of cerebellar ataxia with bronchial asthma. Patient had a history of febrile convulsion at 6 years of age. His family history revealed that his father has hypertension and brother and sister have diabetes mellitus.

On examination of CNS, patient was unable to walk, had slurred speech, waddling gait with wide steps and intention tremors were present. He was unable to write. SPECT scan of the brain showed moderate hypoperfusion in left fronto-parietal and left temporal regions. Moderate to severe hypoperfusion was seen in bilateral cerebellar regions (Figure 1a).

Patient was given hESC as primary treatment. The patient has taken hESC therapy on regular schedules since 2012. Following the treatment, the patient showed improvement in hand eye coordination, could stand without support and increased his muscle strength in all the limbs. The patient was able to walk 50 to 55 steps without support and able to turn while standing without support. His speech was remarkably clearer than before, power in hands increased with hand grip tighter than before, handwriting became clear and tremors reduced as compared to pretreatment status. The patient stated good energy levels and the reduction in twitching of cheek muscle. Minimal hypoperfusion in bilateral cerebellar regions and nearly normal cereberal perfusion was noted on SPECT scan imaging done after the patient received hESC therapy (Figure 1b). The patient has been followed up in November 2014 and is keeping well.

Case 3

A 41-year female was admitted to Nutech Mediworld on 1 February

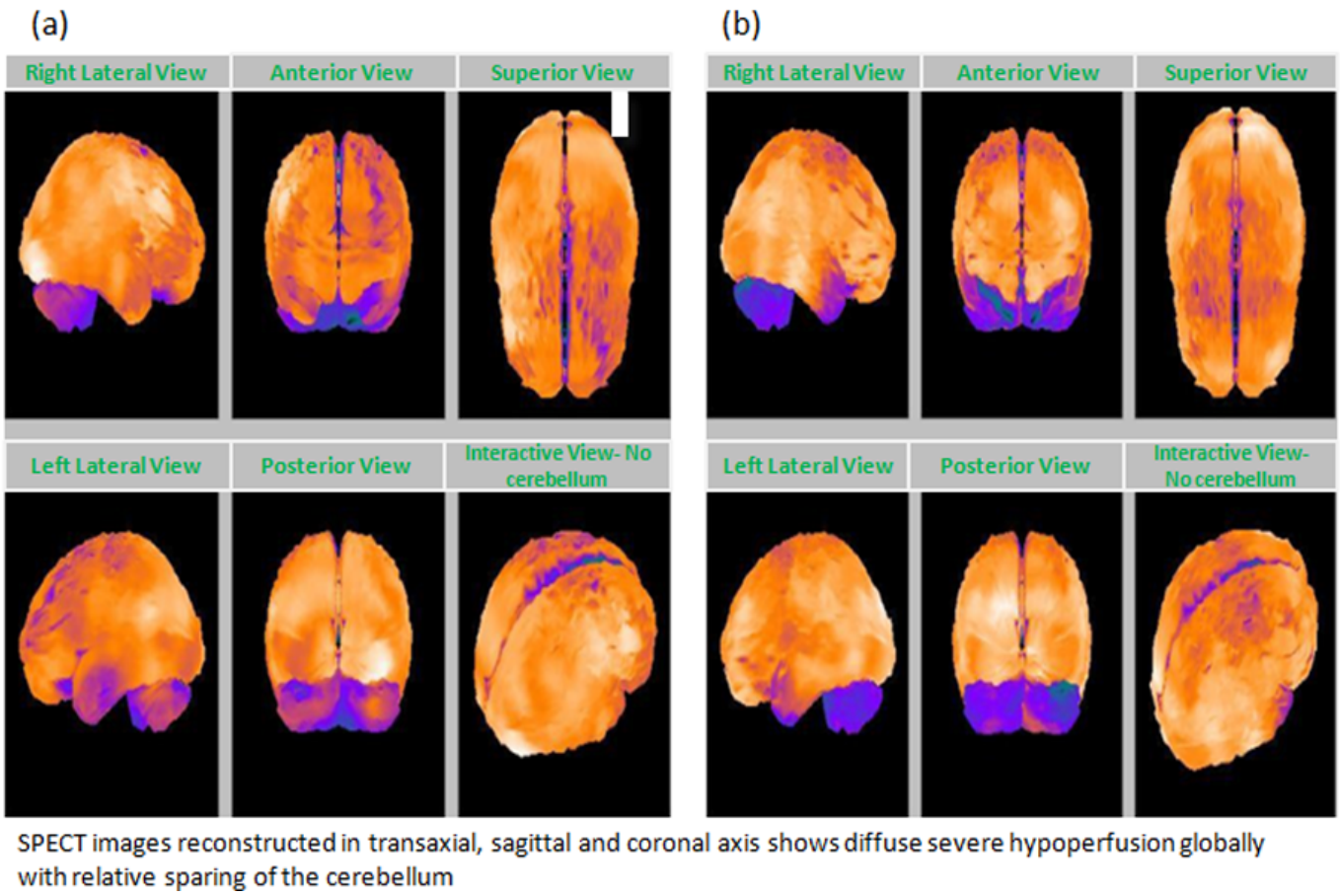

Figure 1(a-b): SPECT Scan Images Before Therapy.

2012 with complaints of tremors of the upper limbs (ULs), unsteadiness of gait, walking imbalance, slurred speech and difficulty in negotiating narrow passages.

The patient was apparently well 13 years back when she noticed some involuntary movements in bilateral ULs. The symptom gradually worsened with time. She complained of tremors in ULs. She finds it difficult to hold the pen and there was deterioration in handwriting. Patient reported wide base gait with ataxia and found it difficult to negotiate narrow passages. She needed complete assistance for walking. The initiation of movement was jerky and there was slowness of the movement. The patient had a pain in UL and lower limb (LL) due to fatigue. The speech was slow and unclear. Other symptoms include fatigue and tiredness.

Patient was a known case of spinocerebellar ataxia. Her past history revealed that she was hypertensive. Patient had a strong family history with mother, grandmother and uncle with the same disorder.

On examination of CNS, the patient was unable to walk without support, had slurred speech and wide base gait. The patient showed presence of ataxia, bradykinesia and plantar extensor. Her bladder control was also affected. SPECT scan of the brain showed moderate to severe hypoperfusion in bilateral cerebellar regions. It also showed mild hypoperfusion in bilateral temporal regions (Figure 2a).

Patient was given hESC as primary treatment along with extensive physiotherapy. After the treatment, patient was able to walk independently (only few steps). She showed improvement in the strength of bilateral UL and LLs, balancing, stamina, endurance and coordination. The patient had better control over trunk and legs following the treatment. SPECT scan of the brain after the patient received hESC therapy showed minimal hyproperfusion. (Figure 2b).

Discussion

Our study reported the use of in-house cultured hESC therapy in the treatment of three patients with SCA. The three patients had different levels of injuries and suffered from cerebrospinal ataxia. All the patients had difficulty in walking, difficulty in speaking and had bladder dysfunctions. After regular sessions of hESC therapy at our institute, these patients showed signs of improvement in their health such as improvement in strength of limbs, ability to stand or walk, reduction in tremors and clearance in speech. The patients also showed improvement in their stamina, endurance and coordination. No adverse events (AEs) were reported. The case of patient 1 is peculiar as her twin sister who suffered from the same ailment did not receive the treatment and eventually died. Her other family members also suffered with SCA . We did SPECT scan imaging of all the three patients to detect the change in degree of hypoperfusion in cerebral and cerebellar regions, following the hESC therapy. Significant decrease in the degree of hypoperfusion was noted in all the patients. No teratomas were seen in patients following the treatment with hESC. We cannot deny that these patients are still not fully cured of their ailment but the improvement

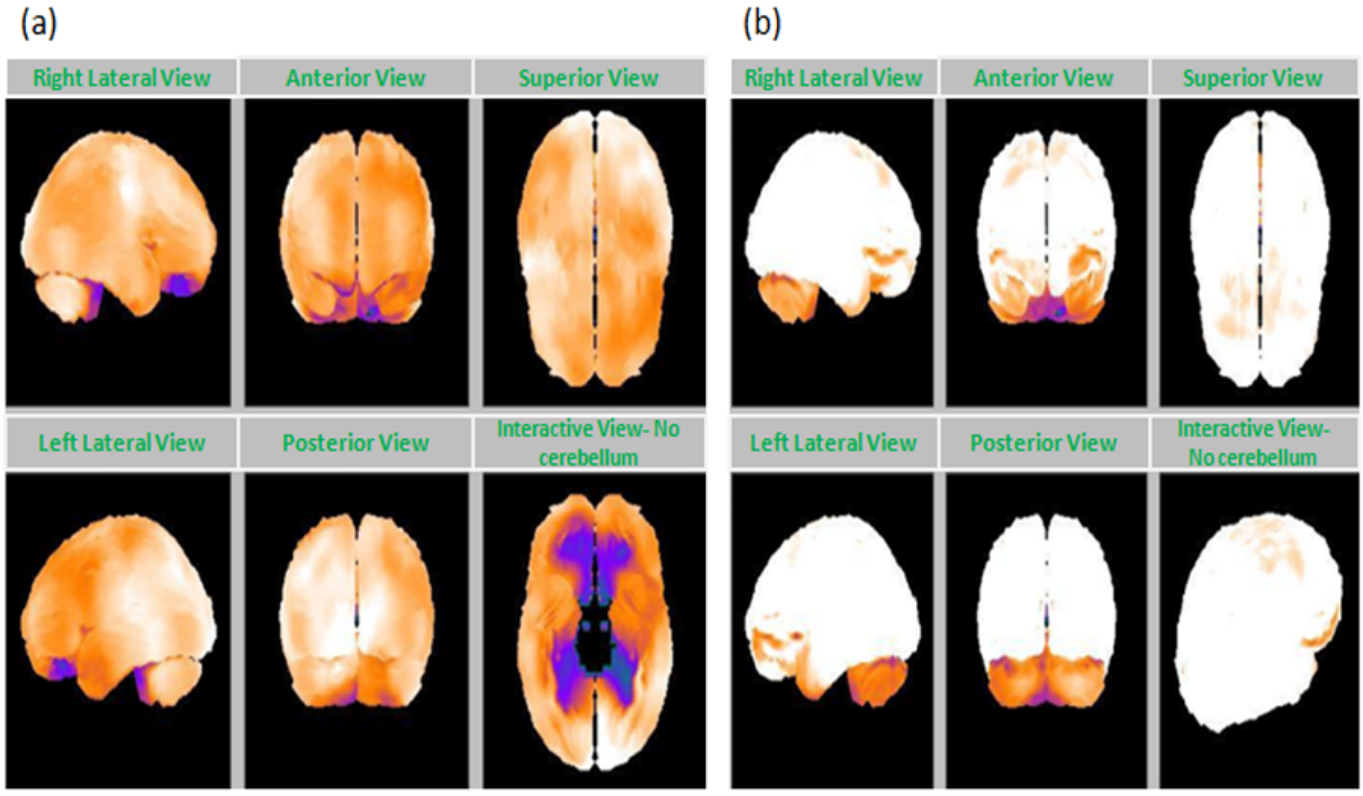

Figure 2(a-b): SPECT Scan Images After Therapy.

that they have shown has positively affected their quality of life and also reduced dependence on caretakers.

Calatrava-Ferreras et al. [5] conducted an *in vitro* study in which they administered HuUCBMCs through i.v route in the 3-acetylpyridine rat model of cerebellar ataxia. HuUCBMCs were found to promote the activation of microglial cells in brain stem by reducing the loss of neurons. A significant improvement in motor coordination was observed [5]. Jin et al. used HUCMSCs to treat 16 patients with SCA. The study observed that treatment with HuUCMSCs resulted in relieving of symptoms for up to 6 months. Follow-up data of one year indicated no immunological reactions and AEs in the patients [12].

Erceg et al. [8] were the first to show that hESCs could be differentiated into neurons and could express similar markers to the developed human cerebellum. Implantation of these differentiated hESCs, transfected with MATH-1 green fluorescent protein into neonatal mice resulted in migration of these cells across the molecular and the purkinje cell layers and settlement in the internal molecular layers. No teratoma formation was observed in the mice [8].

Previously MSCs have been described to possess the potential to migrate to the site of injury, engraft and disseminate through the brain and release tropic factors such as increasing vascular endothelial growth factor and insulin-like growth factor-1 that might stimulate survival of injured neuronal cells and functional recovery [13]. Nakamura et al. [14] in their review of mechanism of action of MSCs on SCA stated that the therapeutic action of MSCs might be due to the secretion of innate factors to induce neural growth and synaptic connection and reduce apoptosis. In other words, the MSCs reach the site of injury and help repair the affected tissue [14]. So we might possibly explain that hESCs used in our study also rely on the same mechanism of action.

At a molecular level, it is well known that genetic mutations play an important role in SCA and autosomal dominant cerebellar ataxia is caused by a polyglutamine-coding tri-nucleotide (CAG) repeat expansion in the coding region of the ATAXN2 gene. This polyglutamine toxicity has been associated as a causative factor of SCA rather than loss of ataxin-2. Xia and colleagues developed an *in vitro* model of SCA type 2 using human induced pluropotent stem cells and observed that the CAG repeats of SCA2 were stable throughout reprogramming and neural differentiation and that these affected cells were short lived [15]. Though we have not studied our patients at a molecular level, we might assume that the normal hESCs in our study patients replaced the short lived affected neuronal cells and also helped in correcting the expansion mutations to normal repeats. In a recent study on transcriptomic analysis of SCA revaled that about 40% of the SCA genes are most highly expressed in the cerebellum and most of the genes have high variable expressions in the entire brain regions [16]. When we analyzed our patients through SPECT scan after the therapy, the hypoperfusion at the cerebral region was reduced as compared with the initial SPECT scan at the start of the therapy.

Conclusion

No study till date assessed the therapeutic potential of hESCs-derived cells in the treatment of patients with SCA. Our study is the first to use hESCs in restoring neurological function of patients with SCA. However, more clinical trials and follow-up studies are needed to prove the long term efficacy and safety of hESCs in the treatment of patients with SCA.

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