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Human Umbilical Cord Mesenchymal Stem Cells for Severe Neurological Sequelae due to Anti-*N*-Methyl-D-Aspartate Receptor Encephalitis: First Case Report

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Abstract

Anti-*N*-methyl-D-aspartate (NMDA) receptor encephalitis is caused by altered patient immune reactions. This study reports the first patient with severe neurologic sequelae after NMDA receptor encephalitis treated with allogeneic umbilical cord–derived mesenchymal stem/stromal cells (UC-MSCs). A 5-year-old girl was diagnosed with NMDA receptor encephalitis and treated with immunosuppressive medicaments and intravenous immunoglobulin (IVIG). Despite intensive therapy, the patient's condition worsened so that allogenic UC-MSC therapy was contemplated. The patient received three intrathecal infusions of xeno- and serum-free cultured UC-MSCs at a dose of 10^6 cells/kg. At baseline and after each UC-MSC administration, the patient was examined by the German Coma Recovery Scale (CRS), the Gross Motor Function Classification System (GMFCS), the Gross Motor Function Measure–88 (GMFM-88), the Manual Ability Classification System (MACS), the Modified Ashworth Scale, and the Denver II test. Before cell therapy, she was in a permanent vegetative state with diffuse cerebral atrophy. Her cognition and motor functions improved progressively after three UC-MSC infusions. At the last visit, she was capable of walking, writing, and counting numbers. Control of urinary and bowel functions was completely recovered. Cerebral atrophy was reduced on brain magnetic resonance imaging (MRI). Overall, the outcomes of this patient suggest a potential cell therapy for autoimmune encephalitis and its neurological consequences.

Keywords

autoimmune encephalitis, anti-NMDA receptor encephalitis, neurological sequelae, mesenchymal stem cells, mesenchymal stromal cells

Introduction

Anti-*N*-methyl-D-aspartate (NMDA) receptor encephalitis is brain inflammation caused by autoimmune antibodies targeting the NMDA receptor on the surface of neurons[1]. Treatment of autoimmune encephalitis (AE), including anti-NMDA receptor encephalitis, includes corticosteroids and intravenous immunoglobulins (IVIGs) and/or plasma exchange followed by other immunosuppressants in refractory cases[2]. Although the response rate of patients to those treatments is generally high, 50% of patients still have cognitive and behavioral problems, 33% have ongoing seizures, and approximately 2% of patients suffer from severe disability. For patients with anti-NMDA receptor encephalitis, only 29% of cases achieve full recovery[3].

Recently, stem cell therapy has emerged as a new therapeutic option for many diseases. Bone marrow–derived mononuclear cell and mesenchymal stem/stromal cell (MSC) infusions are safe and might improve clinical outcomes in patients with various neurological conditions[4–8]. MSC administration was also applied for AE. Bone marrow–derived MSCs were able to inhibit pathogenic T-cells and B-cells, prevent demyelination, increase axonal density, and protect oligodendrocytes from apoptosis in mouse models of

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autoimmune encephalomyelitis. As a result, the disease severity was reduced, and the survival rate was increased in treated mice[9,10]. In humans, administration of autologous adipose-derived stromal vascular fraction improved daily activities in all six treated patients with autoimmune refractory epilepsy and decreased epilepsy frequency in three cases[11].

In this article, we report the first case of a study describing a child who recovered from severe neurological sequelae due to anti-NMDA receptors after three infusions of allogeneic UC-MSCs.

Methods

Umbilical Cord Donor Screening

Mothers who fulfilled the following inclusion criteria: (1) older than 18 years old; (2) in a good health condition; (3) full-term pregnancy; (4) giving birth via cesarean; (5) allowing umbilical cord collection after birth; (6) agree to donate UC-MSCs for research AND was not subject to any exclusion criteria: (A) having critical health problems; (B) experienced pregnancy-related complications or illness; (C) having an acute bacterial or fungal infection or blood infection; (D) positive for Hepatitis B virus (HBV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), herpes simplex virus (HSV), syphilis or chlamydia or other sexually transmitted diseases; and (E) suffering from malaria, rabies, Creutzfeld-Jacob disease, or Chagas disease.

Isolation and Culture of UC-MSCs

The umbilical cord was collected directly after birth in 0.9% sodium chloride (Bidiphar, Vietnam) and transferred to a cell processing center. UC-MSCs were isolated under xeno-free and serum-free conditions as described previously[12]. Briefly, the cord was washed in phosphate buffered saline (PBS) and 70% alcohol and then cut into small pieces. The mixture was digested in 500 U/ml collagenase (Gibco, Grand Island, NY, USA) at 37°C using a GentleMACS Dissociator (Miltenyi, Germany). Cells were filtered and suspended in PowerStem MSC1 Medium (PAN Biotech, Germany) (donor 1) or StemMACS™ MSC Expansion Media XF (Miltenyi, Germany) (donor 2) supplemented with 100 U/ml Pen/Strep (Life Technologies, USA). They were seeded in treated cell culture flasks (NUNC Thermo Scientific, Rochester, NY, USA), coated with CellStart™ substrate (Thermo Fisher Scientific, Grand Island, NY, USA) and cultured at 37°C under 5% CO_2. The cells were harvested once they reached 80% confluency. The cells were passaged for subsequent culture at a cell density of 4,000 cells/cm^2 and quality characterization or cryopreserved in CryoStor® CS10 (Stem Cell Technology, Canada) in the gas phase of liquid nitrogen in an automated Brooks System (Brooks Life Science, Chelmsford, MA, USA) to maintain the temperature at –196°C.

Characterization of UC-MSCs

UC-MSCs were tested for sterility, marker expression, viability, population doubling time, colony forming unit (CFU) potential, multilineage differentiation, karyotype, and potency as described in detail previously[12]. Briefly, cell supernatant was tested for bacterial and fungal infections in the Diagnostic Department at the Vinmec Times City International Hospital, Hanoi, Vietnam, using a BacT/Alert®3D microbial detection system (Biomerieux, Durham, NC, USA). Mycoplasma was tested by a MycoAlert™ Plus Mycoplasma Detection Kit (Lonza, Switzerland) and measured using a Lucetta Luminometer (Lonza, Switzerland) following the manufacturer's instructions.

The expression of the positive markers CD73, CD90, and CD105 and the negative markers CD34, CD45, CD11b, CD19, and HLA-DR (human leukocyte antigen–DR isotype) was analyzed using a Human MSC Analysis Kit (Becton Dickinson, Franklin Lakes, NJ, USA) and Navios and BD Canto II flow cytometers (Beckmann Coulter, Indianapolis, Indiana, USA, and Becton Dickinson, Franklin Lakes, NJ, respectively).

Differentiation assays were performed using a StemPro™ Osteogenesis, Adipogenesis, and Chondrogenesis Differentiation Kit (Gibco, Grand Island, NY, USA) according to the manufacturer's instructions. The cells were stained with Alizarin Red S, Oil Red O, and Alcian Blue (Sigma, Singapore) to detect the osteogenic, adipogenic, and chondrogenic lineages, respectively.

The karyotype analysis was performed in a KaryoMax Colcemid solution (Gibco, Grand Island, NY, USA) to arrest cells in metaphase. The cells were then fixed with Carnoy's fixative and stained with Giemsa (Merck, Germany), and metaphase cells were examined using a Metaphase System and Ikaros software (Karl Seizz, Germany).

The immunomodulatory potency of the UC-MSC line was analyzed in a coculture assay using UC-MSCs and peripheral blood mononuclear cells (PBMCs) derived from six different healthy donors. PBMCs were stained with carboxyifluorescein diester amine (CFSE) (Invitrogen, Eugene, OR, USA) before coculture to evaluate their proliferation capacity. The cells were incubated in RPMI supplemented with 10% fetal bovine serum (FBS, Life Technologies, Grand Island, NY, USA), Pen/Strep (Life Technologies, Grand Island, NY, USA), and 1% phytohemagglutinin (PHA, Life Technologies, Grand Island, NY, USA) to activate T-cells. After 4 days, the cells were stained with anti-CD3 VioBlue antibody (clones: BW264/56) and 7-AAD (Miltenyi, Germany) and analyzed with a BD Canto II flow cytometer (Becton Dickinson) and FlowJo software. PBMCs cultured in the absence of PHA and PBMCs activated with PHA without UC-MSC coculture were used as the negative and positive controls, respectively.

Preparation of UC-MSCs for Cell Therapy

For cell therapy, UC-MSCs were cultured in the xeno-free and serum-free culture conditions described above without antibiotic supplementation and cryopreserved at passage 3. Prior to the intervention, UC-MSCs were thawed and cultured until passage 6. The cells were prepared in 10 ml of 0.9% NaCl (B. Braun, Vietnam) at a dose of 1×10^6 cells/kg patient body weight for the infusions. Cell numbers, viability, MSC marker expression, sterility, and endotoxin levels were determined. The release criteria of cell therapy products were as follows: (1) free of bacteria, fungi, and mycoplasma; (2) viability $\geq$ 90%; (3) UC-MSC markers fulfilled the minimum criteria for MSC according to ISCT 2006[13]; and (4) product was within endotoxin limits for intrathecal route of application, that is, 0.2 EU/kg/h. The cell product was transported to the ward and stored at room temperature until use.

Intervention

In accordance with the patient's severe condition, the potential risks and benefits of cell therapy and intrathecal infusion were explained to the parents in detail. Upon obtaining written informed consent and approval from the Hospital's Board of Directors, UC-MSC therapy was applied. The cells were infused intrathecally through the space between the fourth and fifth lumbar vertebrae using an 18-gauge needle within 30 min under general anesthesia as described previously[6]. Three infusions were performed on April 4, 2019, December 6, 2019 (8 months after the first infusion), and June 10, 2020 (6 months after the second infusion). The patient was followed up until 28 months after the first infusion and 14 months after the last infusion.

Outcome Measurements

Outcomes were assessed using the modified Rankin Scale (mRS)[14], the German Coma Recovery Scale (CRS)[15], the Gross Motor Function Classification System (GMFCS)[16], the Gross Motor Function Measure–88 (GMFM-88)[17], the Manual Ability Classification System (MACS—link https://www.macs.nu/)[18], the Modified Ashworth Scale for muscle spasticity[19], and the Denver II test[20].

For each assessment, an experienced physical therapist and a pediatrician independently rated the outcome measures. The assessments at the baseline and follow-up visits were performed by the same experts to reduce personal errors.

Results

Characterization of UC-MSC Lines

The quality of the second UC-MSC line was assessed (Fig. 1). Both cell lines at passages 3 and 6 expressed high levels of MSC markers, including CD90, CD73, and CD105, and were negative for negative markers, including CD45, CD34, CD19, CD11b, and HLA-DR, as recommended by ISCT[13] (Fig. 1A). The cells showed a comparable population doubling time from passage 2 to passage 6, suggesting their active proliferation state during the analyzed period (Fig. 1B). The population doubling time ranged from 17.53 $\pm$ 1.43 h to 22.17 $\pm$ 0.55 h. For comparison, the average population doubling time of 29 analyzed UC-MSC samples in our histological cohort was 21.73 $\pm$ 1.77 h[12]. Furthermore, UC-MSCs formed high numbers of colonies with an average of 462.96 CFUs $\pm$ 139.81 CFUs per 1,000 cells at passage 3 and 342.59 CFUs $\pm$ 16.04 CFUs per 1,000 cells at passage 6. A representative colony is depicted in Fig. 1C. The CFU numbers of this line were similar to those of 29 analyzed UC-MSC samples at passage 3, with an average $\pm$ SD of 366 CFUs $\pm$ 154 CFUs per 1,000 cells[12]. UC-MSCs were differentiated into osteogenic, adipogenic, and chondrogenic lineages and incubated with Alizarin Red S, Oil Red O, and Alcian Blue to detect calcium deposition, lipid drops, and proteoglycans, respectively. The differentiated cells showed positive staining with the dyes, as depicted in Fig. 1D. Moreover, the cells were able to inhibit the proliferation of CD3+ T-cells derived from healthy donors (n = 5) (Fig. 1E). The UC-MSCs showed a normal karyotype (Fig. 1F).

The UC-MSC line obtained from the first donor was analyzed and reported previously[21]. The cells exhibited normal MSC marker expression. The population doubling time was 28 $\pm$ 1.3 h, and the colony frequency was 140 CFUs $\pm$ 15 CFUs per 1,000 cells. The cells were capable of differentiation into osteogenic, adipogenic, and chondrogenic lineages. Furthermore, cells maintained a normal karyotype after a culture period of six passages.

Quality Control of the Cell Therapy Product

UC-MSCs were cultured until passage 6 or 5 for the first and two other infusions, respectively, and were tested for viability, expression of MSC markers, sterility, and purity (Table 1). The cells showed more than 90% viable cells; a normal MSC phenotype; negative bacterial, fungal, and mycoplasma results; and less than 0.05 EU/ml endotoxin.

Patient Report

Medical History Before Cell Therapy

The child developed normally during the first 58 months until suffering from convulsions and weakness of the left arm and foot on August 10, 2018. On electroencephalogram (EEG), slow delta waves with a frequency of 2 to 3 kHz were observed in the right hemisphere of the brain. Epilepsy was diagnosed, and the patient was treated as an outpatient at the National Children Hospital on August 10, 2018. The patient was hospitalized on August 22, 2018, due to headaches, vomiting, irritability, aphasia, and screaming. Examination on admission showed that movements of the left arm and foot decreased, the tendon reflexes increased, and bilateral Babinski reflexes were present. There was no fever, fecal or

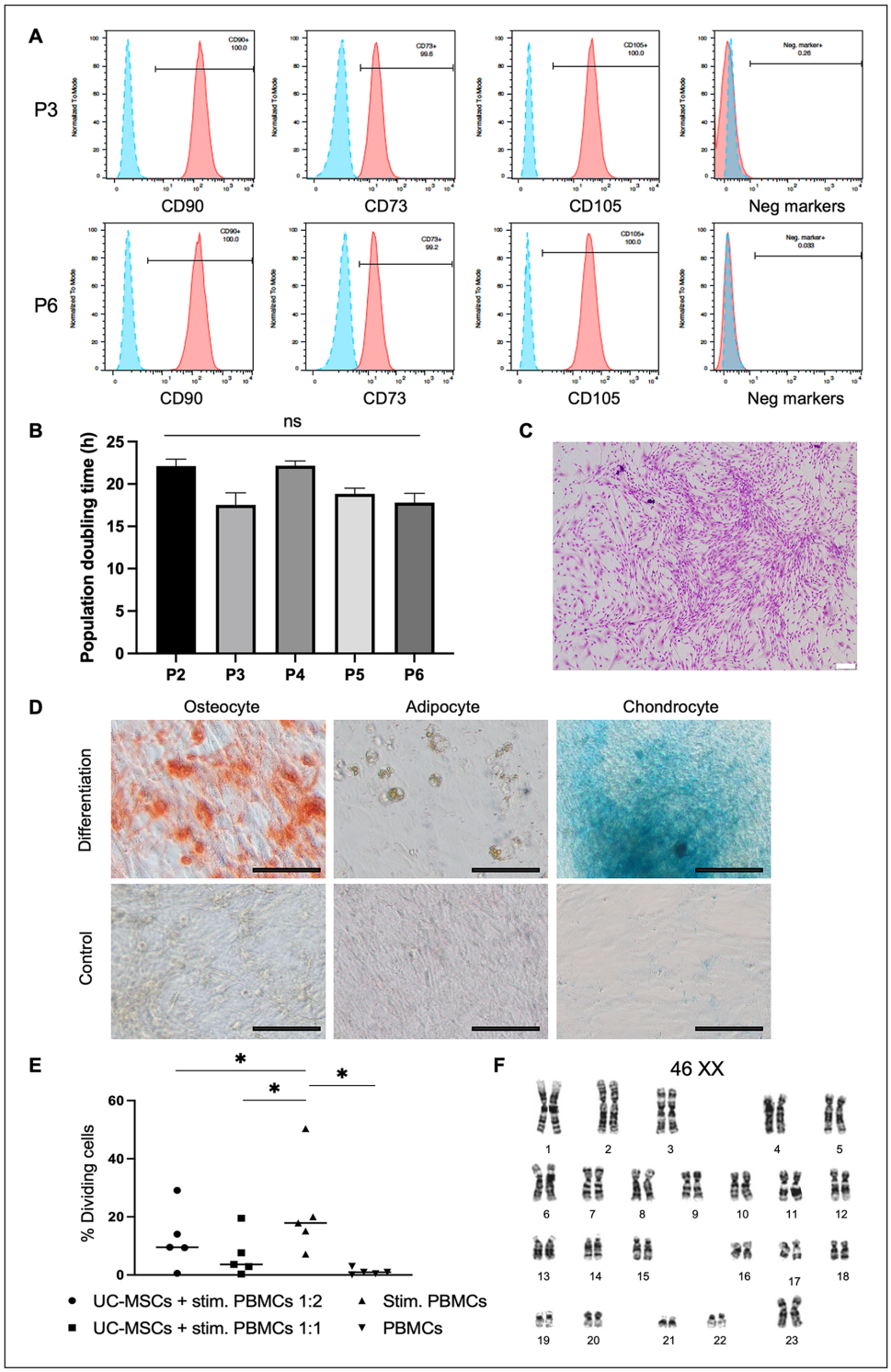

Figure 1. Characterization of the administered UC-MSCs. (A) UC-MSCs showed high expression of CD90, CD73, and CD105 and low levels of negative markers (CD45, CD34, CD19, CD11b, and HLA-DR). The continuous line represents marker expression levels of samples of interest; the dashed line depicts the corresponding isotype controls. (B) The UC-MSC line indicated a comparable population doubling time from passage 2 to passage 6. (C) The cells formed large colonies in the colony forming assay. The white bar scale represents 100 µm. (D) They were capable of multilineage differentiation into osteogenic, adipogenic, and chondrogenic lineages. The black bar scale represents 50 µm. (E) Functional test to analyze the immunomodulatory potential of the UC-MSC line on CD3+ cells derived from peripheral blood of healthy donors ($n = 5$). (F) The karyotype of a representative cell is depicted. The UC-MSCs showed a normal karyotype with 46 XX. UC-MSC: umbilical cord–derived mesenchymal stem/stromal cell; HLA-DR: human leukocyte antigen–DR isotype; PBMC: peripheral blood mononuclear cell.

Table 1. Therapeutic Cell Products.

	The first treatment (April 2019)	The second treatment (December 2019)	The third treatment (June 2020)
Donors	Donor 1	Donor 2	Donor 2
Infused passage	6	5	5
Number of trans-planted cells (dose)	17×10^6 cells/kg	19×10^6 cells/kg	22×10^6 cells/kg
Viability	95%	98%	92%
MSC markers (negative markers include CD45, CD34, CD19, CD11b, and HLA-DR)	CD73: 99.5%, CD90: 100%, CD105: 100%, Negative markers: 0.8%	CD73: 99.94%, CD90: 99.98%, CD105: 99.63%, Negative markers: 1.48%	CD73: 99.96%, CD90: 99.97%, CD105: 96.36%, Negative markers: 0.04%
Bacteria and fungi	Negative	Negative	Negative
Mycoplasma	Negative	Negative	Negative
Endotoxin	$<$0.05 EU/ml	$<$0.05 EU/ml	$<$0.05 EU/ml

MSC: mesenchymal stem/stromal cell; HLA-DR: human leukocyte antigen–DR isotype.

urinary incontinence, or sensation disorder. A complete blood count showed white blood cells of 17.43 G/l, red blood count of 4.68 T/l, 70.5% neutrophils, 21.3% lymphocytes, 6.3% monocytes, 0.1% eosinophils, and 0.3% basophils. Cerebrospinal fluid (CSF) analysis exhibited a protein concentration of 0.18 (g/l), glucose of 4.74 (mmol/l), and an absence of nucleated cells. Japanese encephalitis virus, herpes simplex virus, and enterovirus were negative.

The patient was diagnosed with AE based on positive screening of anti-NMDA receptor antibodies in cerebral spinal fluid on August 22, 2018. Treatment was initiated with solumedrol 20 mg/kg/day for 5 days followed by prednisolone at 30 mg/day, decreased by 10 mg every 7 days and lasting for 16 days; IVIG 400 mg/kg/day for 7 days; depakine 300 mg/day; and risperidone 1 mg/day. From August 27, 2018, she suffered from myoclonus, muscular hypertonicity of both the upper and lower extremities, constipation, and urinary incontinence.

From September 1, 2018 onward, myoclonus progressed, and the patient became unresponsive so that oral feeding through a nasogastric tube was needed. Treatment was switched to cellcept 500 mg per day from September 11, 2018. Topamax 2 mg/kg/day was indicated on September 12, 2018, and increased to 3 mg/kg/day after 10 days. However, none of the symptoms, including myoclonus and unconsciousness, improved. Rituximab 375 mg/m^2/day was given once per week between September 24, 2018, and October 24, 2018, resulting in reduced myoclonus, but general muscular spasticity increased, and unconsciousness persisted. The patient was discharged on October 22, 2018, and received acupuncture for 2 months without any improvement.

She was readmitted to National Children Hospital on December 27, 2018. An examination indicated complete loss of awareness, intense muscle spasticity of the whole body, and intermittent seizures. Feeding was maintained through an oral nasogastric tube. Tocilizumab was infused once per month at a dose of 8 mg/kg for 2 months. Cellcept 250 mg/day, depakine 150 mg/day, and keppra 500 mg/day were given for 2 days as inpatients and then continued as outpatients, but her condition remained unchanged.

Supplemental Table S1 depicts the medications and duration of the therapy in detail.

Evaluation Before Cell Therapy

The patient was admitted to Vinmec International Hospital on March 26, 2019 (7 months after the onset of the illness). Her body weight was 17 kg. She suffered from a severe disability, and her mRS score was 5 (Table 2). Her awareness was completely lost, with a German CRS score of 6 points (Table 2). Intermittent seizures were observed. The GMFCS was scored at level V, the GMFM-88 at 23 points, and hand function was poor with a MACS at level V. Increased muscle tone was observed with the Modified Ashworth Scale of two points for the upper and lower limbs (Table 3). Feedings were maintained through the nasal gastric tube because she could not swallow. Urinary incontinence and constipation were noted. Personal-social, fine motor, language, and gross motor abilities according to Denver II were severely impaired (Table 4). Brain magnetic resonance imaging (MRI) revealed diffuse cerebral atrophy in the supratentorial region, dilatation of the third ventricle and bilateral lateral ventricles (Fig. 2A).

Allogeneic UC-MSC Infusion and Improvements

In accordance with the patient's severe condition, the parents were explained in detail the potential risks and benefits of cell therapy and intrathecal infusion. Upon obtaining written informed consent and approval from the Hospital's Board of Directors, UC-MSC therapy was applied.

The first infusion was performed on April 4, 2019, with 17 million UC-MSCs. The characteristics of the MSC product are presented in Table 1. No severe adverse events occurred during or after the procedure, and the patient was discharged 48 h after the infusion. Medication was continued with risperidone 1 mg/day, keppra 500 mg/day, and piracetam 800 mg/day. Daily physical therapy was performed at home by the patient's mother.

Table 2. Changes in the Modified Rankin Scale and the German Coma Remission Scale.

	Before the first treatment (March 2019)	After the first treatment (November 2019)	After the second treatment (June 2020)	After the third treatment (October 2020)
Modified Rankin Scale	5	5	4	3
German Coma Remission Scale				
Arousability/attention	2	2	5	5
Motor response	3	3	6	6
Response to acoustic stimuli	0	1	2	2
Response to visual stimuli	1	1	2	3
Response to tactile stimuli	0	1	2	3
Logomotor (speech motoric) response	0	2	2	2
Total	6	10	19	21

Table 3. Changes in Motor Functions.

	Before the first treatment (March 2019)	After the first treatment (November 2019)	After the second treatment (June 2020)	After the third treatment (October 2020)
GMFCS	V	V	IV	I
GMFM-88				
Lying and rolling	17	18	51	51
Sitting	6	6	48	60
Crawling and kneeling	0	0	4	42
Standing	0	0	3	37
Walking, running, and jumping	0	0	0	65
Total	23	24	106	255
MACS	V	V	II	I
Modified Ashworth Scale	Score 2 for both upper and lower extremities	Score 2 for the upper and score 1 for the lower extremities	Score 0 for the upper and score 1 for the lower extremities	Score 0 for both upper and lower extremities

GMFCS: the Gross Motor Function Classification System; GMFM-88: the Gross Motor Function Measure–88; MACS: Manual Ability Classification System.

At re-examination on November 26, 2019 (7 months 22 days after the first cell infusion), the patient's body weight increased to 19.5 kg. Muscle spasticity and dysphasia were reduced. Nasogastric tube feeding was discontinued, and the patient was switched to normal oral feeding. The mRS score was 5 (Table 2). The patient was able to react to external stimuli with a German CRS of 10 points (Table 2). Motor functions showed no significant change; both GMFCS and MACS remained at level V, and GMFM-88 scored 24 points. Muscle spasticity measured 2 points in the upper extremities and 1 point in the lower extremities (Table 3). Better head and neck control was also observed. The patient was able to turn to the sides. Denver scores remained unchanged (Table 4). Constipation and urinary incontinence persisted.

The second infusion of UC-MSCs was safely carried out on December 6, 2019, with 19 million UC-MSCs (Table 1). The patient was discharged after 2 days and then continued to receive risperidone 1 mg and keppra 500 mg per day and daily physical therapy at home. The doses of risperidone and keppra were reduced gradually and discontinued 1 month after the second infusion without the manifestation of epilepsy.

Re-examination on June 9, 2020 (14 months 5 days after the first MSC therapy) showed a reduced mRS to 4 and an improved awareness with a German CRS of 19 points (Table 2). The patient' gross and fine motor skills resulted in better scores in all analyzed tests: the GMFCS was reduced to level IV, GMFM-88 was increased to 106 points, and MACS was improved to level II. Muscle spasticity was reduced from 2 points to 0 points for the upper limbs and remained at 1 point for the lower limbs (Table 3). The patient was able to sit and use her hands to pick up foods and other objects. Denver II scores also showed improvement in all areas, including personal-social, gross motor, fine motor-adaptive, and language (Table 4). However, urinary incontinence and constipation remained unimproved.

The third administration of 22 million UC-MSCs was performed without side effects on June 10, 2020 (Table 1), followed by daily physical therapy at home after discharge.

Re-examination 4 months later (18 months after the first infusion) revealed that the patient's disability was reduced to moderate with a mRS of 3 (Table 2). The patient's awareness further improved, with a German CRS of 21 points

Table 4. Changes in Denver II Tests.

Denver II test	Before the first treatment (March 2019)	After the first treatment (November 2019)	After the second treatment (June 2020)	After the third treatment (October 2020)
Personal—Social	0.5	0.5	3	10
Fine motor—Adaptive	0.5	0.5	7	15
Language	0.5	0.5	6	6
Gross motor	0.5	0.5	6	24

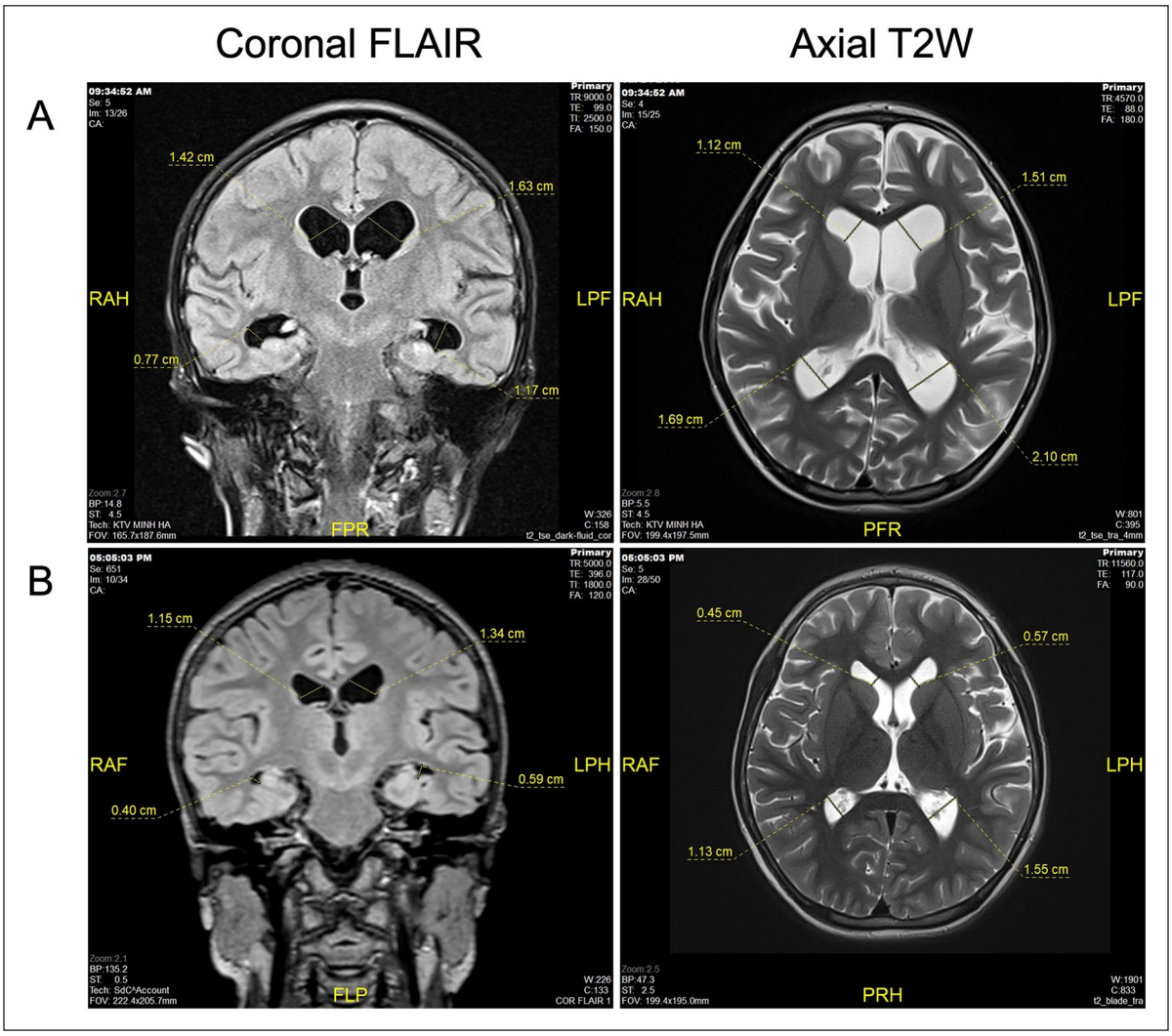

Figure 2. MRI images of the patient before the treatment and after three UC-MSC infusions. (A) Brain MRI before the first transplantation showed diffuse cerebral atrophy and dilatation of the third and bilateral ventricles. (B) Improved brain MRI images with milder dilatation of the lateral ventricles, subarachnoid and sulcus were observed after three UC-MSC administrations. MRI: magnetic resonance imaging; UC-MSC: umbilical cord–derived mesenchymal stem/stromal cell.

(Table 2). Examination of motor function indicated GMFCS at level I and GMFM-88 scores of 255 points. MACS was at level I, and muscle tone reversed to normal with the modified Ashworth scale at score 0 (Table 3). She could walk normally, eat independently, and practice writing. Significant improvements were also observed in Denver II tests (Table 4). Urinary incontinence and constipation were slightly improved. Brain MRI indicated that cerebral atrophy was remarkably reduced with mild dilatation in the left lateral ventricle (Fig. 2B).

Three months later (21 months after the first infusion), she could draw, write, and speak some words. Her urinary

and fecal function was completely controlled. In the last examination (28 months after the first infusion, 14 months after the third infusion), she could count numbers and be prepared for school. The mRS score was 1.

Discussion

For patients with severe psychoneurological sequelae after AE, there is currently no effective treatment. Our patient received two lines of medication and rehabilitation, but all interventions failed to improve her condition. The patient was in a vegetative state when she was first admitted to Vinmec International Hospital and received cell therapy as a last resort. The patient's cognition and motor skills recovered progressively following UC-MSC infusions. After the second administration, she was able to sit up, and after the third infusion, the patient could walk normally without any assistance. In addition, hand skills progressed significantly. At the last check-up, the child could do all daily activities and practice writing and drawing. Language bounced back, the epilepsy disappeared, and no medications were further needed.

Zappia et al. performed intravenous administration of MSCs isolated from C57BL/6J mice to treat autoimmune encephalomyelitis. They found that MSC infusion before disease onset reduced disease severity. They suggested that inhibition of T-cells could be the underlying mechanism of infused MSCs[22]. Zhang et al. demonstrated the potential of human bone marrow MSCs to induce functional recovery in a mouse model of autoimmune encephalomyelitis. MSCs increased axonal density, inhibited demyelination, and protected oligodendrocytes from apoptosis. As a result, mice infused with MSCs showed improved survival and reduced disease severity and relapse rates[9,23]. Gerdoni et al.[10] also reported that MSCs can effectively reduce relapsing-remitting autoimmune encephalomyelitis and prevent late motor disability in animals by inhibiting pathogenic T-cells and B-cells. Our observation was in line with previous findings, in which UC-MSCs were able to suppress T-cell proliferation. Improvements were also observed on cerebral MRI, in which cerebral atrophy was reduced remarkably after three UC-MSC infusions.

To the best of our knowledge, this is the first patient with severe neurological sequelae following anti-NMDA receptor encephalitis whose motor functions and cognitive behaviors recovered after intrathecal infusions of UC-MSCs. We chose UC-MSCs because they have several advantages over other sources. These cells are easy to harvest and expand in culture[24]. Moreover, they are an ideal choice for allogenic use due to their low immunogenicity and strong immunomodulatory potency. Through the secretion of a number of cytokines, such as prostaglandin E2, indoleamine 2,3-dioxygenase, interleukin-10, interleukin-4, TGF-β, and TNF-stimulated gene 6 protein, UC-MSCs can inhibit the proliferation of T-cells, B-cells, and NK-cells and promote the macrophage M2 anti-inflammatory phenotype[25–28]. UC-MSCs can also protect and regenerate nerve cells in a paracrine manner. They release many proangiogenic and neurotrophic factors, such as hepatocyte growth factor, fibroblast growth factor, vascular endothelial growth factor, nerve growth factor, brain-derived neurotrophic factor, and glial cell-derived neurotrophic factor, to stimulate angiogenesis and neurogenesis[29–31].

In a study with 51 children suffering from anti-NMDA receptor encephalitis in Central South China, 14% of patients had severe deficits during a median follow-up period of 16.1 months (range: 1–47.8 months). The median time from therapy to full recovery was 4 months (range: 0.9–12 months)[32]. Titulaer et al. reported that 57% of 221 patients who received first-line and second-line immunotherapies recovered, while almost half of the patients had a mRS score above 3 at a median follow-up of 24 months after disease onset. The most significant improvement was observed in the first 8 months. The outcome of patients with a mRS score above 5 tended to be better within 18 months but worse at the 24-month follow-up[33]. A retrospective study was performed with 71 children with AE who were treated with immune therapy at the Vietnam National Children Hospital. An mRS score less than 3 was observed in 28 of 71 patients (39.44%), 51 of 70 patients (72.86%), 53 of 63 patients (84.13%), and 44 of 51 patients (86.27%) at follow-up visits after 1, 3, 6, and 12 months, respectively (unpublished data). The data indicated the most significant improvement of the analyzed patients within the first 6 months.

The patient in our study was refractory to two lines of immunotherapy in the first 7 months after the diagnosis. She then received three infusions of UC-MSCs and experienced motor and cognitive recovery until the last examination 28 months after the first cell therapy and 35 months after disease onset. It remains to be further investigated whether the recovery of the patient was due to cell therapy or to its natural improvement after immunotherapy. Therefore, randomized controlled clinical trials are necessary to confirm the findings of this individual case and to elucidate any questions that remain.

Conclusion

Our case report suggests that UC-MSC therapy may ameliorate severe neurological sequelae due to anti-NMDA receptor encephalitis. A study with a larger sample size should be performed to evaluate the efficacy of UC-MSCs for AE as well as severe neurological sequelae due to AE.

Authors' Note

We confirmed that the manuscript has been read and approved for publication by all the authors. A preprint manuscript is available: Nguyen LT, Hoang VT, Thu HL, Nguyen PAT, Hoang DM, Ngo

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Author Contributions

L.N.T.: conception and study design, administrative support, provision of study material and patients, performing clinical assessments and follow-ups of the patient, data analysis and interpretation, manuscript writing, and final approval of manuscript. V.T.H.: provision of stem cell processing and characterization, data analysis and interpretation, manuscript writing, and final approval of manuscript. H.L.T.: performing clinical assessments and follow-ups of the patient, data analysis, manuscript writing, and final approval of manuscript. P.A.T.N.: performing UC-MSC infusion, patient care during and after the intervention, and final approval of manuscript. D.M.H.: provision of stem cell processing and characterization, manuscript revision, and final approval of manuscript. D.V.N., H.C.V., and V.N.T.B: collection of data, data analysis, and final approval of the manuscript. M.H.: data analysis, manuscript revision, and final approval of manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by the Vinmec International Hospital's Board of Directors and the Research Institute for Child Health, Vietnam National Children's Hospital, Ethics Committee (No. VNCH-RICH-2019-40).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with Vinmec International Hospital's Director Board and the Research Institute for Child Health, Vietnam National Children's Hospital, Ethics Committee's (No. VNCH-RICH-2019-40) approved protocols.

Statement of Informed Consent

Informed consent was obtained from a legally authorized representative before cell therapy for the interventions and for anonymized patient information to be published in this article.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: L.N.T., V.T.H., H.L.T., P.A.T.N., D.M.H., and D.V.N. are employed by the not-for-profit Vinmec Healthcare System. M.H. declared an advisory role for Regenerative Medicine at Vinmec Healthcare System.

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Supplemental Material

Supplemental material for this article is available online.

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