Mesenchymal stem cell application in children with subacute sclerosing panencephalitis

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ABBREVIATIONS

MSC	Mesenchymal stem cell
SSPE	Subacute sclerosing panencephalitis
SSS	Subacute sclerosing panencephalitis scoring system
WeeFIM	Functional Independence Measure for Children

Subacute sclerosing panencephalitis (SSPE) is a serious, often fatal disease that responds poorly to current treatment modalities. Recently, the ability of mesenchymal stem cells (MSCs) to produce neurotrophic factors and inflammatory molecules has placed them among potential treatment agents for neurological conditions. We report the results of four patients treated with MSC for SSPE. The patients were followed up clinically, and by periodical laboratory evaluations, magnetic resonance imaging (MRI), and electroencephalography. One patient deteriorated to stage III of the disease, two patients remained in the same stage, and one died from disease progression and respiratory problems. Neurological findings and electroencephalography scores were consistent with the clinical course of the patient whereas MRI showed new inflammatory lesions in two patients. This is the first report of the application of MSC in SSPE. No benefit is demonstrated.

Subacute sclerosing panencephalitis (SSPE) is a chronic disorder caused by persistent measles virus.[1] Treatment options are scarce, and results, variable. Neuroprotective approaches have been suggested because neuronal death and demyelination contribute to neurological deterioration.[2] Mesenchymal stem cells (MSCs) capable of improving neuronal survival have been tried in experimental allergic encephalomyelitis: proliferative response against myelin antigens and neuronal death decreased, resulting in clinical improvement.[3] Autologous MSCs alleviate safety and immunocompatibility concerns, and have been tried in phases II and III in neurological disorders including stroke and multiple sclerosis.[4] The 'orphan disease' status of SSPE, its downhill course, and the absence of definitive treatment make it a candidate for MSC transplantation trials aiming at local immunomodulation and neuroprotection.

METHOD

Five patients with SSPE whose disease progressed despite at least 3 months' routine treatment with inosiplex and antiepileptics were eligible for MSC infusion after parental request between January 2009 and June 2012 (Table I). Special approval was obtained from ethical boards of Hacettepe University, Ankara, and the Ministry of Health, Turkey (2011, number 48873). Patients were at stage II or stage III of the disease. One was excluded from the study because post-MSC evaluations could not be performed: under intermittent respiratory assistance for the previous 3 months, he died from respiratory problems 5 weeks after the first MSC infusion and before any follow-up visit. The remaining four patients are presented here.

Patient 1 received 11 intravenous and eight intrathecal MSC infusions at 2- to 8-month intervals over 3 years. Patients 2 and 3 received two intravenous and intrathecal applications at 2-month intervals. Patient 4 received only one intravenous and intrathecal MSC application.

Autologous bone-marrow-derived MSCs were grown under good manufacturing practice at ATI Technology (Karadeniz Technical University-ATI Technology Stem Cell and Gene Therapy Center, Turkey) and Acıbadem Cell Laboratory and Cord Blood Bank (Acıbadem Hospital, Turkey) and enriched for 4 to 6 weeks. MSCs were administered by rapid intravenous infusion (1×10^6 to 2.2×10^6 cells per kg in 50mL 0.9% NaCl) and by lumbar puncture (intrathecal; 0.5×10^6 cells in a maximum of 1mL) on the same day.

Clinical evaluation

Neurological examination was performed by a paediatric neurologist (BK) before and every 3 months after MSC infusion. Clinical stages of SSPE were defined as follows:

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Table I: Patients characteristics before and after mesenchymal stem cell (MSC) infusion

Patient	Age (y)	Sex	Stage at diagnosis/stage at MSC application	Course until MSC application	Course after MSC application	Measles antibody synthesis indices	SSS score[a]	Mental age (mo)	EEG scores[b]
1	9	Male	II/III	Stable	Stable	Baseline: 4.1 After first intrathecal application: 5.8 +24mo: 3	Baseline: 48 +3mo: 52 +6mo: 51 +12mo: 58 +24mo: 58	Baseline: 4 +3mo: 2 +6mo: 2 +12mo: 2 +24mo: 2	Baseline: 6 +3mo: 6 +6mo: 6 +15mo: 7 +24mo: 7
2	11	Male	I/II	Progression	Progression, then died	Baseline: 3 After first intrathecal application: 3	Baseline: 24 +2mo: 28	Baseline: 24 +2mo: 24	Baseline: 9 +3mo: 4
3	7	Male	II/II	Progression	Progression	Baseline: 3 After first intrathecal application: 3	Baseline: 40 +3mo: 59 +6mo: 56 +12mo: 60 +24mo: 64	Baseline: 24 +3mo: 24 +6mo: 18 +12mo: 1 +24mo: 1	Baseline: 6 +3mo: 4 +6mo: 4 +15mo: 5 +24mo: 6
4	9	Male	II/II	Progression	Progression then motor improvement	Baseline: 3 After first intrathecal application: 3 +24mo: 5.1	Baseline: 18 +3mo: 34 +6mo: 33 +12mo: 35 +24mo: 35	Baseline: 36 +3mo: 24 +6mo: 18 +12mo: 18 +24mo: 15	Baseline: 5 +3mo: 4 +6mo: 5 +15mo: 6 +24mo: 6

[a]SSS-evaluated mental, sensory, and motor functions are rated from 0 (normal) to 80 points (death). [b]EEG score: from 0 (normal EEG) to 10 (maximum score). EEG, electroencephalography; MSC, mesenchymal stem cell; SSS; subacute sclerosing panencephalitis scoring system.

What this paper adds

- Mesenchymal stem cell treatment had no clear effect in patients with subacute sclerosing panencephalitis.
- The treatment may be associated with new inflammatory lesions.

stage I, cognitive and behavioural changes only; stage II, ambulatory (with/without assistance), myoclonia, some verbal communication; stage III: bedbound, some non-verbal response to environmental stimuli. Clinical status was evaluated with the SSPE scoring system (SSS)[1] and mental age was determined in the paediatric developmental unit using the Hacettepe SSPE Short Mental Assessment Scale[5] or Denver II Developmental Test (Turkish standardization) according to the patient's developmental level. A paediatric physiotherapist (MG) evaluated daily activities with the Functional Independence Measure for Children (WeeFIM), scored from 0 to 126 (best performance),[6] functional motor capacity with the Gross Motor Function Measure, where maximum (normal) score is 100,[7] and the Gross Motor Functional Classification System (GMFCS), rated from I (good) to V (worst).[8] An appropriate home programme was given after the physiotherapist's assessment.

Laboratory tests included titres for measles immunoglobulin-γ in serum and cerebrospinal fluid at intervals of 2 to 8 months after the first MSC infusion, magnetic resonance imaging (MRI) at intervals of 4 to 16 months, and electroencephalography (EEG) before and every 3 months after the first MSC infusion. A dedicated neuroradiologist (KKO) noted the presence of new lesions and changes in previous lesions on MRI. EEG recordings were assessed by a paediatric epileptologist (DY) and quantified using a scoring system modified from Ferrillo et al.[9] Background activity, presence and frequency of periodical slow waves, and presence of other epileptic activity were scored separately and total score was recorded. All evaluating specialists were blinded to patients' treatment.

RESULTS

The characteristics of the patients are given in Table I. Three were in stage II with disease progression under inosiplex treatment (patients 2–4) and one in stage III (patient 1).

No acute adverse effects, including fever, rash and itching, or infection, occurred during or after MSC application. Tests of liver and kidney function remained normal. Patient 1 remained in the same stage, patient 2 died from disease progression and respiratory problems 3 weeks after the second MSC infusion, patient 3 progressed to stage III, and patient 4 remained in stage II with fluctuations in SSS (Table I). SSS scores and mental ages during follow-up are shown in Table I and Figure S1 (online supporting information).

Magnetic resonance findings

Patient 1 had widespread atrophy and white matter involvement before MSC application which continued during 4 years' follow-up (Figure S2, online supporting

information). Patient 2 had posterior periventricular white matter and callosal lesions before MSC infusion. Patient 3 showed apparent progression with new cortical–subcortical lesions at 4 months, and necrosis and atrophy at 10 months (Figure S3, online supporting information). Patient 4 had normal MRI before treatment. At 4 months, parieto-occipital cortical T2 hyperintensity, posterior periventricular, callosal, and striatal involvement were observed; at 24 months, striatal atrophy, progression in cortex and white matter lesions (Fig. 1).

Indices of measles antibody synthesis fluctuated between 3.0 and 14.0 during the 3 years' follow-up in patient 1, the last value being 3.0. In the other patients, the index remained stable after the first intrathecal MSC application (Table I). EEG scores followed the clinical state of the patient: stable in patient 1, worsening in patient 2, and mild worsening in patients 3 and 4 (Table I). Scores on the Gross Motor Function Measure, WeeFIM, and GMFCS showed slight improvement in patient 1, deterioration in patient 3, and deterioration followed by improvement to independent ambulation at 24 months in patient 4 (Table SI, online supporting information).

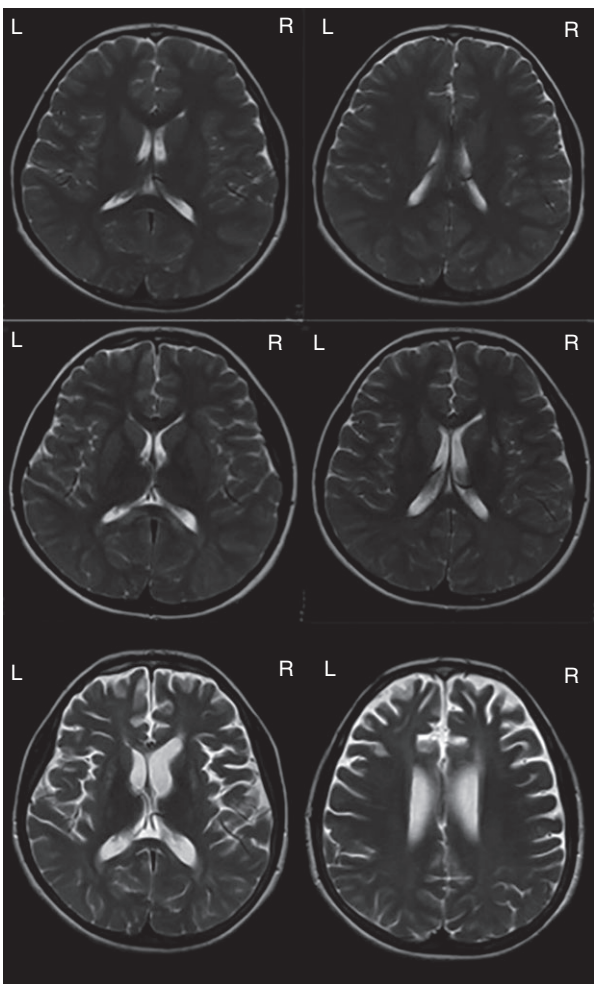

Figure 1: Axial T2-weighted magnetic resonance images of patient 4.

DISCUSSION

This is the first trial of MSCs in SSPE or, to our knowledge, any human chronic viral encephalitis. We treated patients with stage II and III disease who were already worsening under routine treatment. The disease course showed no uniform pattern among patients after MSC infusion. One patient died from disease progression and respiratory problems 3 weeks after the second MSC application. Patients 1 and 3 had better responsiveness according to parental reports. Patient 4 showed a fluctuating course. Interestingly, new inflammatory lesions appeared on MRI in patients 3 and 4 after MSC application, with (patient 4) or without (patient 3) accompanying clinical deterioration. Such lesions are rare during the course of SSPE[10] and may be related to MSCs.

We could have anticipated beneficial effects of MSC in SSPE through neuroprotection in the subacute progressive phase (patients 2–4), or remyelination in the chronic or sequalae phase (patient 1), by supportive action on neurons and oligodendroglia respectively. Most MSC trials in neurological disorders have shown no significant adverse effect and potential benefit.[11] Autologous MSCs are being tried in multiple sclerosis, amyotrophic lateral sclerosis, spinal cord injury, multiple system atrophy, and Alzheimer disease.[4] Expected benefits include reduced inflammation and cell death, stimulation of tissue repair, endogenous neurogenesis, neurite outgrowth, and synaptogenesis. MSCs exert these effects through secretion of soluble cytokines and trophic factors.[4] On the other hand, their secreted cytokines and chemokines may activate existing inflammation. In SSPE, inflammation and oedema tend to occur in stage II, and gliosis in stage III.[10] Our two patients who developed new oedematous lesions were in stage II: existing inflammation may have facilitated the formation of new lesions after MSC treatment, whereas severe atrophy and gliosis in patient 1 might have prevented the manifestation of inflammation on MRI. Direct migration of MSCs into parenchyma can also cause inflamed lesions in experiments where cells are transplanted at high density.[12] Our patients received no intraparenchymal injection and cell density was minimal compared with the above experiments (10^5 cells per hemisphere). However, our discussion is limited by the absence of tissue diagnosis.

Indices of measles antibody synthesis did not show specific patterns during and after treatment. MSCs are immunomodulatory or immunosuppressive in vivo and in vitro: they can induce or prevent differentiation and immunoglobulin secretion of B cells depending on experimental conditions.[13] Measles İmmunoglobulin G titers or synthesis index do not correlate with clinical state in SSPE.[1] Hypothetically, the highly active intrathecal immunoglobulin synthesis of patients with SSPE may not be overcome by MSC application. In patient 1, the increased measles antibody indices 3 months and 6 months after intrathecal

application suggest increased inflammatory response and evoke the inflamed MRI lesions developing at similar time points in patients 3 and 4.

One limitation of our study was the variable application schedule: the injection interval of 2 to 8 months was chosen arbitrarily considering repeated injections would be necessary in a chronic disease, and varied according to the clinical state (intercurrent infections) of patients and the availability of MSC. Optimal treatment times should be discussed, comparing patients with early and late SSPE. In experimental allergic encephalomyelitis, MSCs are effective when given at peak disease, before chronic, irreversible damage begins. On the other hand, MSC transplantation 1 week after spinal injury demonstrates better results than immediate transplantation.[14] In our study MSCs given in stage II produced an inflammatory effect, whereas there was no apparent alteration in stage III where chronic damage and disability were already established.[15]

In this case series, no clear benefit of MSCs in SSPE was demonstrated for objective criteria. Moreover, two children also showed new inflammatory lesions on MRI. The variable course of SSPE, including temporary remissions/fluctuations, is an obstacle when assessing treatment results. Experimental studies of persistent infection of the central nervous system caused by mouse hepatitis virus in mice, or infection of transgenic animals expressing measles virus receptors, may clarify the efficiency and side effects of MSC application and treatment schedule for chronic viral brain infections.

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SUPPORTING INFORMATION

The following additional material may be found online:

Figure S1: Subacute sclerosing panencephalitis scoring system (SSS) scores and mental ages (mo) of patients.

Figure S2: Axial T2-weighted magnetic resonance images of patient 1.

Figure S3: Axial T2-weighted magnetic resonance images of patient 3.

Table SI: Standardized assessments of independent function and motor capacity.

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