Advances in Medical Sciences 62 (2017) 273–279

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Treatment of refractory epilepsy patients with autologous mesenchymal stem cells reduces seizure frequency: An open label study

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ARTICLE INFO

Article history:
Received 28 September 2016
Accepted 21 December 2016
Available online xxx

Keywords:
Mesenchymal stem cells
Epilepsy
Seizure
Cell therapy
Clinical trial

ABSTRACT

Purpose: Existing anti-epileptic drugs (AED) have limited efficiency in many patients, necessitating the search for alternative approaches such as stem cell therapy. We report the use of autologous patient-derived mesenchymal stem cells (MSC) as a therapeutic agent in symptomatic drug-resistant epilepsy in a Phase I open label clinical trial (registered as NCT02497443).
Patients and methods: The patients received either standard treatment with AED (control group), or AED supplemented with single intravenous administration of undifferentiated autologous MSC (target dose of 1×10^6 cells/kg), followed by a single intrathecal injection of neurally induced autologous MSC (target dose of 0.1×10^6 cells/kg).
Results: MSC injections were well tolerated and did not cause any severe adverse effects. Seizure frequency was designated as the main outcome and evaluated at 1 year time point. 3 out of 10 patients in MSC therapy group achieved remission (no seizures for one year and more), and 5 additional patients became responders to AEDs, while only 2 out of 12 patients became responders in control group (difference significant, $P = 0.0135$).
Conclusions: MSC possess unique immunomodulatory properties and are a safe and promising candidate for cell therapy in AED resistant epilepsy patients.

1. Introduction

Epilepsy is a group of neurological conditions, characterized by seizures resulting from excessive and abnormal cortical neural activity, and affecting tens of millions people worldwide [1]. Increasing numbers of antiepileptic drugs and their combinations aim at blocking sodium or calcium channels or other targets to facilitate the termination of epileptic seizures [2]. Since the therapeutic efficacy of anti-epileptic drugs (AEDs) is limited in 20%–40% of patients [3,4], alternatives such as cell therapy are considered a promising approach [5–7]. Stem cell therapy has earlier proven effective in many animal and human neuropathologic contexts [8–12]. The animal model-based body of evidence in favor of stem cell therapy for epilepsy is rapidly accumulating: different types of stem cells, including neural stem cells, embryonic stem cells, fetal progenitor cells, and mesenchymal stem cells, have been used in animal models of epilepsy with some degree of success [13–17]. Therapeutic potential of stem cells can be further enhanced by advanced bioengineering tools such as scaffold encapsulation [18,19], culture regimen adjustments [20–23], and genetic modification [15,24–27].

While the major function of bone marrow mesenchymal stem cells (MSCs) is to support the hematopoiesis and hematopoietic stem cell engraftment, and to supply cells of mesodermal origin such as osteoblasts, chondrocytes, and adipocytes [28], their additional properties include systemic and local immunomodulatory effects and an ability (still debatable) for neural

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http://dx.doi.org/10.1016/j.advms.2016.12.004
1896-1126/

transdifferentiation [29–33], which makes them promising candidates for neurotherapy. In particular, MSC implantation commonly results in the reduction of the number of seizures and better preservation of neurons [14,15,34–40]. Importantly, robust effects of MSC implantation were shown in different rodent models of epilepsy – pilocarpine induction [41] and kindling model of epilepsy [34].

Autologous patient-derived MSC represent a stem cell population easy to isolate and expand without ethical or technological limitations. Building upon extensive and encouraging data from animal studies, and on our previous findings on MSC efficacy in other neurologic patients [42], we are proposing a protocol for the treatment of subjects with drug-resistant symptomatic epilepsy by intravenous and intrathecal MSC infusion. We used autologous stem cells to avoid immune sensitization and rejection of the transplanted cells. The goal of this pilot open label single-center study was to evaluate safety and preliminary efficacy of combined application of autologous MSC and conventional AEDs in human refractory epilepsy patients.

2. Patients and methods

2.1. Trial conduct

This single-center Phase I open label study was initiated at the Republic Mental Health Research Center of Minsk, Belarus, in 2011, with the aim to compare two treatment protocols for refractory symptomatic epilepsy: conventional AED as a control group, and AED supplemented by MSC injections as a cell therapy group. Written informed consent was obtained according to the Declaration of Helsinki from all of the participating subjects. The study design and informed consents were approved by Center's Ethical Committee, and the institutional review board supervised and safeguarded the rights, safety, and well-being of all study subjects. The essential procedures of this clinical trial were one bone marrow extraction and two injections of expanded MSC (one intravenous and one intrathecal). The primary endpoint of the study was safety and tolerability of MSCs injection, and the secondary endpoint was to assess the changes in seizure frequency, EEG parameters and cognitive status after 1 year post-injection. This study was registered with the U.S.A. National Institutes of Health (clinical trial number NCT02497443).

2.2. Subjects

During the period from April 2011 to April 2014, 22 patients were recruited, and all of them remained in the study until completion. Eligible candidates had to meet the following criteria: (1) Males and females aged >18 years; (2) Diagnosed with epilepsy basing on frequent (>5 events per month) seizures, severe post-seizure health states accompanied by cognitive impairment, and behavioral disorders and anxiety; (3) Epilepsy was considered refractory according to the definition of the International League Against Epilepsy, i.e., following the failure of adequate trials of two tolerated antiepileptic drugs (e.g., carbamazepine, valproic acid, topiramate, lamotrigine, and phenobarbital in monotherapy and in different combinations) [43]; (4) No response to AEDs for the previous two years; (5) Candidates have the ability to provide written informed consent. The exclusion criteria were the presence of any concomitant disease that might interfere with the outcome: CNS inflammatory disorders (such as meningoencephalitis of viral or parasitic origin), chronic decompensated psychoses, dementia, CNS tumors, blood test positivity for hepatitis B or C or HIV infection, and/or social disadaptation. The patients were randomized into two groups: (1) standard treatment with AEDs (control group), or (2) AEDs supplemented with single intravenous administration of undifferentiated autologous MSCs (target dose of 1×10^6 cells/kg), followed after 5–7 days by a single intrathecal injection of neuro-induced autologous MSCs (target dose of 0.1×10^6 cells/kg) (cell therapy group).

2.3. MSCs preparation

Autologous MSCs were prepared as described previously [42,44]. Briefly, heparinized bone marrow (BM) was obtained via iliac crest aspiration from the patients. Mononuclear cells were isolated by density gradient centrifugation. The MSCs were selected by plastic adhesion and were immunophenotyped by flow cytometry as $CD90^+CD105^+CD45^-CD34^-$ cells. The MSCs were cultured in a CO_2 incubator (5% CO_2) at 37 °C in α-modified Eagle's medium (α-MEM; Gibco) supplemented with 5% AB human serum, Glutamax and antibiotics. The BM-derived cells were expanded in T175 culture flasks (Sarstedt) at an initial concentration of $3 \times 10^5/cm^2$ (passage 0). When the cells reached 80–90% confluence, they were detached using trypsin/EDTA and then replated at a concentration of 3000 cells/cm^2 (passage 1–2). After two passages, a portion of the MSCs was neuro-induced via culturing for 7 days in Neurocult-XF proliferation medium (StemCell Tech.), as described previously [42,45].

For transplantation, MSCs were harvested, washed by centrifugation and resuspended for injection in saline solution containing 5% autologous serum. The MSC quality control included bacteriological, phenotypical and molecular genetic (i.e., nestin and neuron-specific enolase (NSE) for neuro-induced MSCs) assessments. Cell viability was assessed via the trypan blue exclusion method and was never below 98%. MSC samples were transferred to the clinical center in designated bags. To avoid adverse reactions, $40.0–101.0 \times 10^6$ (mean $68.2 \pm 8.48 \times 10^6$) of the MSCs were resuspended for injection in 20 ml of saline solution containing 5% autologous serum and administered intravenously to the patients over 5–10 min at a concentration of $2.0–5.0 \times 10^6$ cells/ml. Neuro-induced MSCs ($2.7–8.0 \times 10^6$ cells, mean $6.34 \pm 0.72 \times 10^6$ cells) were resuspended in 5 ml of saline solution for injection and administered slowly to the patients between the L3 and L4 spinal segments under local anesthesia with 0.5% Novocaine solution. The short-term safety of the MSC injections was evaluated by medical observations for 1 hour post-injection. Further clinical monitoring of the patients included evaluations of parameters related to the patients' health and behavior states and the frequencies and severities of seizures according to the National Hospital Seizure Severity Scale at 12 months post-therapy [46].

2.4. Clinical assessments

Our main functional outcome parameter was monthly frequency of spontaneous recurrent seizures. To evaluate cognitive impairment, we used the MMSE test [47]. The anxiety and depression states were evaluated using the Hospital Anxiety and Depression Scale [48]. Electroencephalographic (EEG) recordings [49] were performed for each patient prior to the initiation of therapy and at the endpoint of the study using a Mizar EEG-201 encephalograph (Russia) with biopotential registration from 16 body points according to the 10–20 scheme. The observed electrical alpha, beta, delta and theta waves were analyzed in a 3-min segment (with further recalculations every 1 min). The peak alpha wave frequencies were also calculated. Both spontaneous and exercise EEG tests (hyperventilation and photostimulation) were conducted for each patient. We evaluated the quantities of local and generalized epileptiform spikes and waves per minute.