Epilepsy Research 141 (2018) 56–63

Contents lists available at ScienceDirect

Epilepsy Research

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

Intravenous infusion of mesenchymal stem cells reduces epileptogenesis in a rat model of status epilepticus

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ARTICLE INFO

Keywords:
Mesenchymal stem cell
Epilepsy
Cell therapy
Mossy fiber sprouting

ABSTRACT

Objective: Status epilepticus (SE) causes neuronal cell death, aberrant mossy fiber sprouting (MFS), and cognitive deteriorations. The present study tested the hypothesis that systemically infused mesenchymal stem cells (MSCs) reduce epileptogenesis by inhibiting neuronal cell death and suppressing aberrant MFS, leading to cognitive function preservation in a rat model of epilepsy.

Methods: SE was induced using the lithium-pilocarpine injection model. The seizure frequency was scored using a video-monitoring system and the Morris water maze test was carried out to evaluate cognitive function. Comparisons were made between MSCs- and vehicle-infused rats. Immunohistochemical staining was performed to detect Green fluorescent protein $(GFP)^+$ MSCs and to quantify the number of $GAD67^+$ and $NeuN^+$ neurons in the hippocampus. Manganese-enhanced magnetic resonance imaging (MEMRI) and Timm staining were also performed to assess the MFS.

Results: MSC infusion inhibited epileptogenesis and preserved cognitive function after SE. The infused GFP^+ MSCs were accumulated in the hippocampus and were associated with the preservation of $GAD67^+$ and $NeuN^+$ hippocampal neurons. Furthermore, the MSC infusion suppressed the aberrant MFS in the hippocampus as evidenced by MEMRI and Timm staining.

Conclusions: This study demonstrated that the intravenous infusion of MSCs mitigated epileptogenesis, thus advancing MSCs as an effective approach for epilepsy in clinical practice.

1. Introduction

Although there are about 50 millions epilepsy patients in the world (Banerjee et al., 2009), there are limited treatments which including invasive surgery and long term medication which only suppresses the clinical symptoms. Thus, it is important to develop novel approach to treat epilepsy. Temporal lobe epilepsy (TLE) often develops many years after an initial brain insult such as febrile seizure and status epilepticus (SE) (Herman, 2002), which results in cognitive deteriorations (Detour et al., 2005). The recurrent seizure activity may lead to hippocampal damage, which is one of the most common neuropathological findings of TLE (Finegersh et al., 2011). Although the pathophysiology of epileptogenesis following SE is still unclear, the suggested main mechanisms observed in TLE are neural cell loss and aberrant mossy fiber sprouting (MFS) (Dudek and Sutula, 2007).

The transplantation of bone-marrow derived mesenchymal stem cells (MSCs) is suggested to exert therapeutic effects in experimental epilepsy models through the inhibition of neuronal cell loss in the hippocampus (Abdanipour et al., 2011; Costa-Ferro et al., 2012; Costa-Ferro et al., 2010; Huicong et al., 2013; Leal et al., 2014; Li et al., 2009;

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; DH, dentate hilus; DG, dentate gyrus; DMEM, dulbecco's modified eagle's medium; FBS, fetal bovine serum; GAD67, glutamic acid decarboxylase 67; GCL, granular cell layer; GFP, green fluorescent protein; IML, inner molecular layer; MEMRI, manganese-enhanced magnetic resonance imaging; MFS, mossy fiber sprouting; MSCs, mesenchymal stem cells; NeuN, neuronal nuclei; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline-0.1% Tween 20; SE, status epilepticus; SEM, standard error of the mean; SRS, spontaneous recurrent seizures; TLE, temporal lobe epilepsy

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https://doi.org/10.1016/j.eplepsyres.2018.02.008
Received 11 September 2017; Received in revised form 22 January 2018; Accepted 13 February 2018
Available online 14 February 2018
0920-1211/

Long et al., 2013; Venturin et al., 2011; Voulgari-Kokota et al., 2012). However, there are currently limited studies on the suppression of aberrant MFS following MSCs transplantation. Therefore, in this study, we tested the hypothesis that systemically infused MSCs reduce epileptogenesis by inhibiting the neuronal cell death and aberrant MFS, thus resulting in the preservation of cognitive function in an experimental model of epilepsy.

2. Methods

All experiments were performed according to the institutional guidelines of Sapporo Medical University. The animal procedures were approved by the Animal Care and Use Committee of Sapporo Medical University.

2.1. Preparation of mesenchymal stem cells from rat bone marrow

The culture preparation of MSC was based on our previous studies (Morita et al., 2016; Sasaki et al., 2016). Briefly, bone marrow was collected from the femoral bones of adult wild-type and green fluorescent protein (GFP)-expressing Sprague-Dawley rats (W-Tg [CAG-GFP] 184Ys). Subsequently, the material was diluted to 25 ml with Dulbecco's modified Eagle's medium (DMEM; SIGMA, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific Inc., Waltham, MA, USA), 2 mM l-glutamine (SIGMA), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Thermo Fisher Scientific Inc., Waltham, MA, USA), and was incubated for 3 days at 5% CO_2 and 37 °C. When the cultures almost reached confluence, the adherent cells were detached with a trypsin-EDTA solution (SIGMA) and subcultured on 150 mm^2 Tissue Culture Dish (1030-150: IWAKI, Tokyo, Japan; surface area: 148 cm^2) at 5×10^5 cells/ml with 14 ml culture medium; thus, the plating density was approximately $3.4 \times 10^3/cm^2$. A phenotype analysis for the surface antigens $CD45^-$, $CD73^+$, $CD90^+$, $CD106^-$ of the MSCs was conducted. (Kim et al., 2006). The cultured MSCs were used for transplantation after three passages.

2.2. Lithium-pilocarpine model

The lithium-pilocarpine model was used as the epilepsy model and was developed as described previously (Glien et al., 2001). Adult male Sprague-Dawley rats (170–220 g) were intraperitoneally (i.p.) infused with lithium chloride (127 mg/kg in 0.9% NaCl; SIGMA). On the following day, methylscopolamine bromide (1 mg/kg, i.p. in 0.9% NaCl; SIGMA) was administered to limit the peripheral effects of the convulsion. Thirty minutes later, pilocarpine (20 mg/kg, i.p. in 0.9% NaCl; SIGMA) was injected to induce SE (lithium-pilocarpine model). The induced seizure was scored using the Racine scale (Racine, 1972). The rats that manifested both rearing and falling behaviors (Racine scale V) were included in the study as SE. The average latency time to the beginning of the SE was 18.5 ± 1.52 min. The SE was continued for 60 min and terminated by the administration of diazepam (10 mg/kg, i.p.; Takeda, Nagoya, Japan) to reduce the mortality rate.

2.3. Experimental protocol

The experimental protocol is illustrated in Fig. 1. After injecting diazepam, the animals after the SE were divided randomly into the MSC and vehicle groups and were intravenously infused with MSCs (1.0×10^6 cells in 1 ml fresh DMEM) or vehicle alone (1 ml fresh DMEM), respectively, *via* the tail vein one day after the SE induction. Intact Sprague-Dawley rats were used as normal controls. All rats were injected daily with the immunosuppressant cyclosporine A (10 mg/kg, i.p.) (Morita et al., 2016; Nakazaki et al., 2017).

2.4. Evaluation of spontaneous recurrent seizures

The spontaneous recurrent seizures (SRS) were video-monitored (color cameras HDR-CX390 Sony, Japan) from day 21 to day 30 after SE, for 12 h per day (7.00 a.m. to 7.00 p.m.) to determine the epileptic condition (Glien et al., 2001; Lee et al., 2014). Only seizures with Racine scale IV and V scores (Forelimb clonus, rearing, and falling) were counted as previously described (Costa-Ferro et al., 2010; Racine, 1972).

2.5. Morris water maze test

The acquisition of spatial learning was evaluated using the procedures adapted from the Morris water maze test (Iihoshi et al., 2004). The apparatus consisted of an acrylic circular tank (150 cm in diameter) filled with water to a depth of 30 cm, which was maintained at 22–24 °C and made opaque with white tempera paint. The walls of the room contained visual cues that remained constant across the experiments. A clear plastic circular platform (15 cm in diameter) was submerged under 2 cm of water in one quadrant of the tank during all the training trials. Rats were released into the maze head-up and facing the wall of the maze. If an animal failed to find the platform in 60 s, it was placed on the platform for 30 s. All animals were subjected to the same paradigm for 5 days consecutively on days 55–59 after SE. The escape latency was calculated by analyzing the tracks that were recorded through a video camera mounted above the maze and connected to a computerized tracking image analyzer system (ANY-maze, Stoelting CO, IL, USA).

2.6. Immunohistochemical analysis

Rats were anesthetized with ketamine/xylazine (75/10 mg/kg, i.p.) and perfused transcardially with cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The whole brains were dissected, post-fixed in 4% paraformaldehyde overnight, and cryoprotected in 30% sucrose/phosphate-buffered saline at 4 °C. Samples were stored at −80 °C until use. Coronal sections were cut at a 40 μm thickness using a cryostat (Sakura Seiki Co, Tokyo, Japan) at −20 °C. Four sections per animal were selected in total for the analysis, and were cut at 3.6 mm caudal to the bregma according to the rat stereotaxic atlas (Paxinos and Watson, 1998). The sections were washed three times in PBS-0.1% Tween 20 (PBST), blocked in 5% normal donkey serum/0.3% Triton X-100 in PBS at room temperature for 30 min, and incubated in primary antibodies diluted in 5% normal donkey serum/0.3% Triton X-100/PBS at 4 °C overnight. We used the rabbit anti-glutamic acid decarboxylase 67 (GAD67) antibody (1:10000; AB_11070; Abcam, USA) as a marker for gamma aminobutyric acid (GABA) neurons, mouse anti-neuronal nuclei (NeuN) antibody (1:800; AB_177487; Abcam) as a neuronal marker, and chicken anti-GFP antibody (1:2000; AB_13970; Abcam) for the detection of GFP. After washing four times in PBST, the sections were incubated in secondary antibodies, which consisted of goat anti-

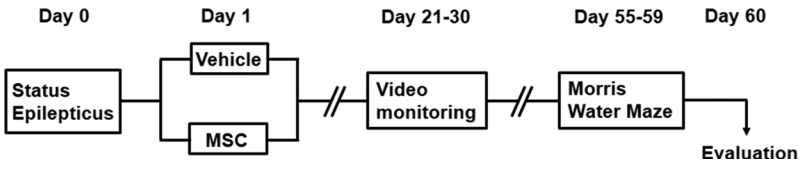

Fig. 1. Timeline of the experiments. One day after establishing the status epilepticus (SE), the rats were intravenously infused with mesenchymal stem cells (MSCs) or vehicle. Video-monitoring was performed between day 21 and day 30 after SE induction to evaluate the seizure frequency. The Morris water maze was performed from day 55 to day 59. The manganese-enhanced magnetic resonance imaging (MEMRI) and histological analyses were performed at day 60.