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Case Series Open Access

Treatment of Lyme Disease with Human Embryonic Stem Cells: A Case Series

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Abstract

Background: Lyme Disease (LD) is a tick-borne disease caused by Borrelia burgdorferi (Bb) and transmitted in humans by Ixodesscapularis. LD can affect any organ of the body. The present study evaluated the efficacy and safety of human embryonic stem cell (hESC) therapy for the treatment of LD.

Methods: Patients included in the present study had experienced symptoms of LD to varying degree of severities and intensities. The study consisted of treatment phases separated by gap phases in between.

Results: Improvement in symptoms was observed after receiving hESC therapy. hESC therapy showed considerable improvement in the condition of the patients who were unable to walk straight or maintain balance while sitting and standing. These patients had regained their balance and had started to perform their regular activities with less effort after receiving hESC therapy. In addition, patients showed improvement in blurred vision, tremors, had higher energy levels, improved stamina, appetite, decreased numbness in the upper limb, decreased stiffness, regained balance, and had no slurring of speech after receiving hESC therapy.

Conclusion: In conclusion, hESC therapy has shown significant improvement in patients with LD. No adverse events were observed in the patients.

Keywords: Human embryonic stem cells; Neuroinfectious disease; Lyme disease

Introduction

Lyme Disease (LD) is a tick-borne disease caused by Borrelia burgdorferi (Bb). Ixodesscapularis, a black legged tick is responsible for transmission of LD in humans and maintenance of the spirochete in the natural reservoir [1]. According to the Centers of Disease Control and Prevention (CDC), the number of LD cases increased from 9,908 cases in 1992 to 19,931 cases in 2006 in the United States [2].

Attachment of Bb on the skin for at least 24 hr after the tick bite results in infection. The length of exposure time decides the severity of the disease [3]. Due to the life cycle of Bb, the symptoms often surface in spring, summer, or early autumn. Most cases with LD present with erythema migrans (bull's eye rash) followed by arthralgia, myalgia, and fever. Although these symptoms are not confirmatory, serological tests [4], neuropsychiatric testing, single-photon emissionctomography (SPECT) scan, magnetic resonance imaging (MRI) brain scans, and cerebro-Spinal Fluid (CSF) analysis are effective tools that may be used to establish the diagnosis of LD [5]. Clinical manifestations in patients with LD include skin, joints, nervous system, and cardiovascular system [6].

There is a lack of defined protocol for the treatment of LD with antibiotics due to uncertain symptoms and clinical manifestations of the disease. According to the International Lyme and Associated Diseases Society (ILADS), no single treatment or medication may result in complete recovery of a patient with LD. There is a need to customize the treatment pattern in patients based on their co-infections, immune deficiencies, and severity of the disease [7]. Although several combination therapies and different treatment regimens have been used in the past for the treatment of LD, there are no studies which have reported treatment with stem cells.

In our previous studies we have shown the improvement in the patient's condition suffering from cortical visual impairment and cerebral palsy after hESCs therapy [8,9]. The present study is the first in our knowledge to evaluate the efficacy and safety of h э лоum five patients. Patients with a previous documented diagnosis of LD or patients who were diagnosed with LD at Nutech Mediworld were included in the study.

The transplantation clinically was under the oversight of an independent ethics committee (IEC). The institutional committee of Nutech Mediworld, a Good Clinical Practice (GCP) certified center for stem cell therapy reported all research with respect to embryonic stem cells to National Apex Body. A written informed consent or video consent was obtained from the patients prior to the study.

Cell culture and differentiation

Two directed cell lines, non-neuronal and neuronal were present in cell culture. The non-neuronal cell lines included progenitor cells for insulin producing stem cells, hematopoietic stem cells, mesenchymal stem cell, hepatocyte stem cell, epithelial stem cells and cardiac stem cells. hESCs in our study were obtained from a single, spare, throwaway, pre implantation stage fertilized ovum taken during natural in vitro fertilization (IVF) process with consent from the donor. The fertilized ovum was suspended in a small amount of minimal essential media and broken mechanically. The product was incubated in carbon-dioxide (CO_2), water jacketed incubator with essential media Dulbecco's Modified Eagle's Medium (DMEM; Himedia Labs, Mumbai, India) and Roswell Park Memorial Institute medium (RPMI; Himedia Labs, Mumbai, India) with addition of β - HCG (16-64 µl of 500 IU/ml, Serum Institute of India, Pune, India) and progesterone (16-64 µl of 250 mg/ml, Sun Pharma, Mumbai, India), in aerobic conditions. After 24 hrs, the product was divided into two different flasks and DMEM and RPMI were added in a ratio of 1:3.5 to 1:35 volume by volume. The cells obtained were re-incubated in a water jacketed incubator at 34-38°C with an atmosphere of 3.5-6% CO_2 for 24 hr in anaerobic conditions. The cells were divided in to three aliquots- one aliquot was reincubated in anaerobic condition with either RPMI or DMEM; second aliquot was stored at freezing temperature and the third aliquot was made ready to injection (RTI) (Patent-WO2007/141657A PCT/1 B 2007; published Dec 2007).

Various marker studies were done to characterize the cells were which include whether the cell were SSEA3+ve; OCT4 +ve; alkaline phosphatase +ve; β actin +ve; NANOG +ve; SOX +ve; β – Nestin +ve; HCG +ve; CD 34 +ve; GAF +ve; NeuN+ve; GATA +ve and TRA –ve. The detailed cell culture and differentiation techniques have been elaborated elsewhere [9].

Name of the drug	Frequency
Monocef injection(1gm)	(bd)
N/S (100ml)	i.v (bd)
Tinidazole (50mg)	(bd)
Minocycline (100mg)	od
Pantop(40 mg)	od
Omez (20 mg)	od
Doxycycline	od
Vizylac/Econorm (Biocodex)	(bd)
Fludac (20mg)	1 cup od
MUI + N/S (10ml)	i.v infusion thrice a week
Rantac	(bd)
Glycopylorate	(bd)
Meganeuron (200 mg)	od
Metoprolol (25 mg)	od
Clonazepam (0.75 mg)	Daily at bedtime
Dilzem SR (120 mg)	od

Study Design

The study consisted of four treatment phases separated by gap phases. The patients were tested for hypersensitivity reactions with hESC (0.25 mL hESC injected subcutaneously). Following safety evaluations, patients entered the first treatment phase (T1, 8 weeks) in which patients received 0.25 ml hESC twice daily intramuscularly to “prime” the body and allow for the recipient immune system not to reject the stem cells and 1 ml hESC twice within 7 days intravenously

along with mild hypoperfusion in the bilateral cerebellar cortices (Figure 1). The MR tractography of the brain performed on 10 Oct 2013 showed interruption of white matter tracts in bilateral anterior and semiovales/corona radiata, progressive paucity of fibers/ tracts was also noted in the brain stream. The patient started to receive hESC therapy as per the Lyme's protocol along with physiotherapy and antibiotics. The patient received 3 sessions (one 8 week session and two sessions of 4 weeks each). After the first session of hESC therapy, the brain SPECT scan showed normal perfusion (Figure 2). The MR tractography showed reduction in the size of several demyelinating plaques in supratentorial and infratentorial compartments with no new demyelinating plaques. The patient was followed up in December 2014 and is well.

Overall after receiving hESC therapy, the patient regained walking balance to a significant extent, showed decreased numbness in the UL, decreased stiffness, regained balance, had no slurring of speech, improved core strength, could write and had good hand muscle strength, and no longer had breathing discomfort.

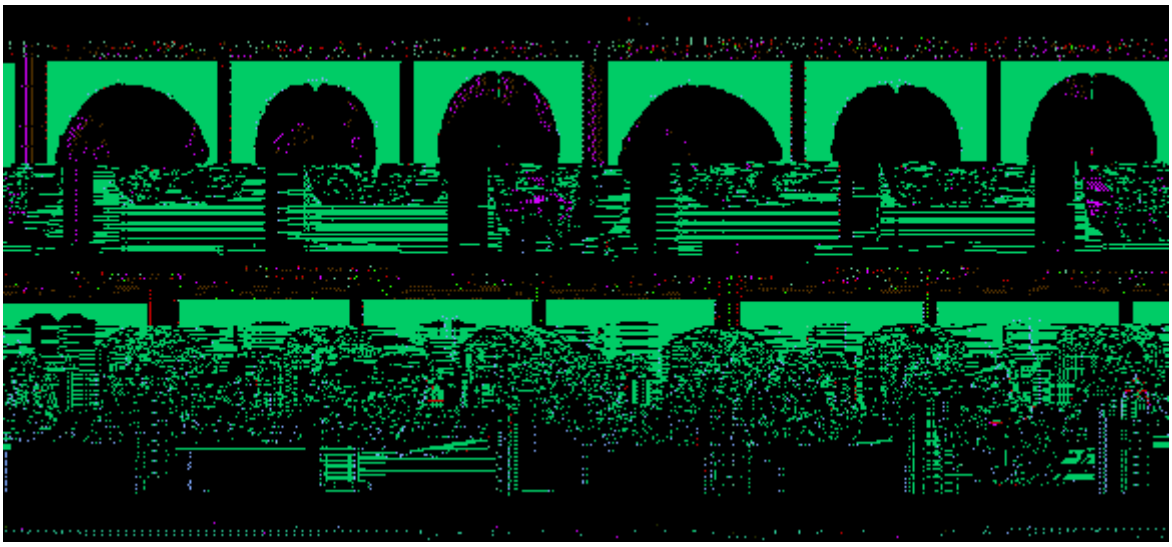

Figure 2: SPECT scan images after receiving hESC therapy showed reduced hypoperfusion(reduced purple areas) (Orange- normal; pink and white- above normal; purple and black- hypoperfused).

Discussion

LD is an inflammatory condition which may result in arthritis symptoms, cardiac problems, facial palsy, viral like meningitis, and neurological problems such as confusion, memory impairment, psychosis, and balance problems [3,10]. The conventional treatment for chronic LD includes antibiotic therapy. Previously conducted studies have shown that antibiotics including ceftriazone, penicillins, tetracyclines, and macrolides are ineffective in the treatment of patients with LD [11]. Although doxycycline has shown effective results in the treatment of early LD, it has not shown any improvement among patients with relapsing or persistent LD [12]. However; most studies suggest that antibiotic therapy is successful in the management of early LD and lack in showing any improvement among patients with persistent/chronic LD. Auwaerter et al showed that