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Human Embryonic Stem Cell Therapy in Crohn's Disease: A Case Report

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Conflict of interest: None declared

Patient: Male, 21
Final Diagnosis: Crohn's disease
Symptoms: Intolerance to specific foods • abdominal pain and diarrhea
Medication: Human embryonic stem cell therapy
Clinical Procedure: Human embryonic stem cell transplantation
Specialty: Gastroenterology

Objective: Unusual or unexpected effect of treatment





Background: Crohn's disease is a chronic inflammatory disease of the intestines, mainly the colon and ileum, related with ulcers and fistulae. It is estimated to affect 565 000 people in the United States. Currently available therapies, such as antibiotics, thiopurines, and anti-tumor necrosis factor-alpha agents, are only observed to reduce the complications associated with Crohn's disease and to improve quality of life, but cannot cure the disease. Stem cell therapy appears to have certain advantages over conventional therapies. Our study aimed to evaluate the efficacy of human embryonic stem cell therapy in a patient with Crohn's disease.

Case Report: A 21-year-old male with chief complaints of intolerance to specific foods, abdominal pain, and diarrhea underwent human embryonic stem cell therapy for two months. After undergoing human embryonic stem cell therapy, the patient showed symptomatic relief. He had no complaints of back pain, abdominal pain, or diarrhea and had improved digestion. The patient had no signs and symptoms of skin infection, and had improved limb stamina, strength, and endurance. The condition of patient was stable after the therapy.

Conclusions: Human embryonic stem cell therapy might serve as a new optimistic treatment approach for Crohn's disease.

MeSH Keywords: Crohn Disease • Inflammatory Bowel Diseases • Stem Cells

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Background

Crohn's Disease (CD) is a chronic inflammatory disease [1,2] estimated to affect 565 000 people in the United States. The pathogenesis of CD is mainly related to the production of interleukin-12 (IL-12), interferon- γ (IFN- γ), and mutations in the gene that encodes NOD2 (nucleotide-binding oligomerization domain 2) protein in a subgroup of patients [3]. CD is also associated with the distribution of human leukocyte antigens (HLA) A and B in the patient. However, the basis of these genetic associations are not clear [4]. Clinical manifestation of CD is associated with inflammatory bowel disease (IBD) and other conditions, such as arthritis, uveitis, sclerosing cholangitis, fever, diarrhea, and mal-absorption, and it is also associated with stricture and malignancy [5–7].

CD affects the quality of life and has a significant functional and psychological impact [8]. Despite advances in medical science, a definitive cure for CD is still not available [9]. The available therapies targeted at managing CD aim at reducing complications associated with CD and ensuring a better quality of life (QoL). Recently, stem cell therapy has been used in CD and has shown several advantages over conventional therapies [10–12].

We have already published studies in which human embryonic stem cell therapy (hESC) was used in treatment of cerebral palsy, cortical visual impairment, Friedreich's ataxia, Lyme's disease, and spinal cord injury [13–17]. Here, we report the use of hESC therapy to treat a 21-year-old man with CD associated with intolerance to specific food items, recurring and constant diarrhea, abdominal pain, recurrent skin infection, lethargy, weakness, and intermittent back pain.

Case Report

A 21-year-old male was admitted at Nutech Mediworld in July 2011 with chief complaints of intolerance to specific food items, abdominal pain, and diarrhea during the last six years, as well as recurrent skin infection, lethargy and weakness, weak limbs and muscles, and intermittent back pain. The patient was intolerant to most food items, e.g., eggs, bread, dairy products, wheat, barley, oats, pastas, cherries, onion, garlic, tea, coffee, honey, and spicy, oily, and fatty food at the time of admission. The patient also showed irregular bowel habits and unpredictable appetite. He was not able to function well in activities of daily living. The terminal ileum showed circumferential wall thickening and reduced distensibility, and it also involved the ileocecal junction. There was no significant proximal small bowel dilatation. The patient came to us from abroad with an already-established diagnosis of CD in 2005. Colonoscopy done on 27 March 2005 showed chronic inflammation at the

mucous membrane of terminal ileum accompanying acute activity inflammatory necrotic tissue and local lymphadenosis. In April 2005, granulomatous terminal ileitis and lymphatic colitis consistent with CD were observed during histopathological examination (HPE). A barium meal examination conducted on 11 April 2005 also confirmed CD with findings of terminal ileitis. The patient was followed-up again in his home country in 2008. His endoscopy showed minor erosion. The colonoscopy done on 17 November 2008 showed terminal ileitis with minor erosion. A repeat HPE done on 24 November 2008 showed mild terminal ileum and chronic inflammation. The cecum and anal canal were normal. The laboratory parameters were within normal ranges.

The patient was prescribed corticosteroids. He had also taken some herbal and ayurvedic formulations. The patient was finally advised to have surgery in his home country. He was admitted to our center on 11 July 2011 when all the treatment modalities had failed, which also included immunomodulation. Contrast-enhanced computed tomography (CECT) done on 16 July 2011 and showed multifocal non-contiguous wall thickening in the terminal ileum extending up to 3.0 cm and ileocecal interception. These findings were compatible with CD.

The patient provided written informed consent prior to the start of treatment. The patient had to undergo a detailed examination by the doctors before, during, and after each treatment cycle. The biochemical investigations were done before the start of the treatment and then at regular intervals. The patient was also reviewed by an external physician. The observations documented by the various teams were then examined by a separate team of physicians not involved with patient care.

At Nutech Mediworld, the patient was given hESC therapy as a primary treatment for two months. The cells are cultured and maintained as per our proprietary in-house technology (Patent-WO 2007/141657A PCT/1B 2007 Published 13 Dec 2007) laboratory with good manufacturing practices, good laboratory practices, and good tissue practices certification. The cell lines are free of animal products and are chromosomally stable. The details of the technique and cell characterization have been described elsewhere [18]. The evidence for the use of hESCs at Nutech Mediworld has also been submitted in writing and was accepted at the House of Lords, Regenerative Medicine, Science and Technology Committee [19].

The patient was initially tested for hypersensitivity reactions with hESC (0.05 ml hESC injected subcutaneously). The patient was then given different doses of hESC that were administered through different routes, including 0.25 mL (<4 million cells) hESCs administered intramuscularly (*i.m.*) daily, 2 mL hESCs (<32 million cells) administered two times a week through intravenous (*i.v.*) route, 1mL hESCs (<16 million cells)

Table 1. Bowel habits and limb movement of the patient before and after the hESC therapy.

Parameters	Before treatment			After treatment		
Bowel habits						
Frequency of stool	4 to 6 times a day			Once daily		
Consistency of stool	Watery stools			Semi solid		
Mucus	Mucus presents			Nil		
Mucus threads (Microscopic examination)	Increased amount of stool			Nil		
Limb movement						
Limbs	TOP/AOP	Left	Right	TOP/AOP	Left	Right
Upper Limb	4" above TOP	11.5"	11"	5" above TOP	11"	11"
	3" below TOP	10"	10"	3.5" below AOP	10"	10"
Lower Limb	4" above AOP	17"	17"	7" above TOP	17.6"	17.6"
	4" below AOP	12.5"	12.5"	6" below AOP	13"	13"

were administered once a week (*i.v.*), and 0.25mL were administered daily via oral drops.

During the study period, the patient's diet and bowel habits were strictly followed.

After two months of hESC therapy, a remarkable systematic relief was observed in the patient (Table 1). At discharge, he had no complaints of back pain, abdominal pain or diarrhea and had better digestion. He had no signs and symptoms of skin infection. The patient had improved limb strength, stamina, and endurance. He was able to eat all food types. The endoscopic findings and ultrasound of the abdomen were normal. His CECT done on 30 August 2011 showed the size of wall thickening involving the terminal ileum, extending from a length of 1.5 cm (which was 3 cm on 16 July 2011). The ileocecal interception was also resolved. After stool analysis, pus cells were found to be 2–3/HPF and epithelial cells were 1–2 HPF. No cyst/ova/bacteria or RBC were found in the stool analysis. On 29 December 2011, no stricture or obstruction was seen in his CECT. The laboratory parameters had no significant change and were within normal range.

He again visited our facility on 23 June 2014 for booster shots. He was not taking any medicines and was clinically normal. A computed tomography (CT) scan of the patient showed no evidence of free fluid, loculated fluid collection, or lymphadenopathy.

Discussion

We used an in-house cultured hESC without any xeno-products. All the media used to culture hESC are free from animal contaminants and cells of animal origin. The harvested hESCs are generated in a culture at the pre-blastocyst stage. The cell line thus developed is created from a single fertilized ovum 24–48 h after fertilization when the conceptus is assumed to have reached the 4–16 cell stage [18].

We observed symptomatic relief as well as radiological changes in the patient following the treatment with hESC. A gradual improvement in food and bowel habits was observed. He was able to eat all food types, was clinically better, and was able to carry out all the activities of daily living. CD is a body-wide disease and has manifestations apart from the digestive system, such as weakness. Our patient initially had weakness in the limbs and lack of stamina. Overall improvement in stamina, endurance, and limb strength was observed after the hESC therapy. Frequency of defecation was once daily and no mucus or mucus thread was observed in the stool.

CD and ulcerative colitis (UC) are incurable diseases, which share common features in pathogenesis but present different clinical pictures. There are several treatment options for CD, such as IL-2 [20], Ustekinumab-human monoclonal antibody [21], and anti-tumor necrosis factor (TNF), but these options have no greater value for curing CD and they increase the postoperative complications [22]. A meta-analysis showed that the immunosuppressive agents (anti-TNF and corticosteroids) used for the management of CD are associated with

post-operative complications such as wounds and septic shock [23]. Although the anti-TNF treatment has been shown to be successful for the treatment of CD patients, the esophagogastroduodenal endoscopy (EGD) findings have shown that “bamboo joint-like appearance” (BJA) of the stomach does not change [24]. Treatment with anti-TNF factor is also limited, as approximately one-third of patients have not been able to respond to this therapy [11].

The concomitant use of immunomodulators like thiopurines, azathioprine, methotrexate, and cyclosporine and biologics like anti-TNF monoclonal antibodies is limited by loss of efficacy in long-term use [25]. In most of these cases, surgical resection is required, but that too has a high recurrence rate [26,27].

Stem cells are known to act by modulating endogenous mesenchymal stem cells (MSCs) and macrophages by secreting chemokines such as transforming growth factor beta (TGF- β) and prostaglandin E2 (PGE-2) hormone. Further, these cells activate T-cells and lead to tissue regeneration and trigger an immunosuppressive response [28]. Treatment with several types of stem cells, such as MSCs [11], hematopoietic stem cells (HSCs) [29], adipose tissue-derived stem cells (ASCs) [30], and allogeneic hematopoietic stem cells [31], have shown favorable therapeutic outcomes, good tolerability, and good safety profile in patients with CD. Daniel et al. reported that HSCs can be used as an alternative therapy for the treatment of CD in patients showing resistance to other therapies, but these cells were difficult to immobilize [29]. Lee et al. conducted a study on 33 patients to evaluate the efficacy and safety of ASCs in the treatment of Crohn's fistula, and reported complete recovery in 26 patients within 12 months but recurrence of disease was observed [30]. Forbes et al. conducted a trial on 15 patients to evaluate the efficacy of allogeneic MSCs in patients with luminal CD and reported that these cells reduced the CD activity index (CDAI) and CD endoscopic index of severity (CDEIS) scores in patients [32]. Cho et al. observed that 50% of the patients showed complete healing when treated with 2×10^7 cells/ml ASCs after a single one-time injection. Further, ASCs may be effective for the treatment of fistula in patients who were unable to achieve complete healing by conventional medical or surgical interventions [33].

The fear of using hESC is largely due to the adverse events (AEs), like teratoma formation and immune rejection. However,

we have not observed teratoma formation in any of our 1400 patients in more than 14 years of use of this hESC therapy. Minor AEs, like injection site reaction, fever, and headache, have been observed in some of our patients [34,35]. However, no AE was reported in our patient with CD. No immunosuppressants or steroids were given to the patient. This might be because the hESCs used in our study are derived from a two-cell staged embryo, post-pronuclear fertilization, which is of human origin. They have not acquired antigenic properties up to this time and are thus non-immunogenic. Further, these cells are free from any animal products and are thus easily accepted by the human body.

hESCs act by repairing and regenerating the injured tissue. Previous studies have shown that stem cells communicate with damaged cells, and home to the site of injury. Homing signals including cytokines, chemokines, and growth factors released at the injured site that attract systemically or locally administered stem cells to the site. These signals function by inducing upregulation of selectins and activation of integrins, present on the stem cells [36–38].

Conclusions

To date, no study has reported the use of hESC in CD. The present study showed that treatment with hESC in CD is safe and well tolerated. The condition of the patient was stable after the treatment with hESCs. This study might provide a new optimistic approach for the treatment of CD. However, further clinical investigations with large sample size are needed for better understanding of the therapy.

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Disclosure

The author declares that she has no conflict of interest.

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