Case report

Clinical translation of the benefits of cell transplantation in a case of cerebral palsy

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A B S T R A C T

Aims: Cerebral palsy (CP) is an umbrella term including a group of permanent disorders of development of movement and of posture causing activity limitation. Oxidative stress and glutamate mediated excitotoxicity are important mechanisms of injury to both the white matter and neurons in the developing brain resulting in motor deficits. No treatment measures are available that can repair the existing damage. Use of bone marrow stem cells for the treatment of CP has shown promise owing to their capacity for self-renewal, differentiation with a potential of neuroregeneration and secretory paracrine effects. Methods: To study the efficacy of cell transplantation in CP, we administered autologous bone marrow mononuclear cells intrathecally to a 2.2-year-old female patient. She was followed up at 9 months and a repeat transplantation was administered. A second follow up was done 5 months after second transplantation. Gross Motor Function Classification System-Expanded & Revised (GMFCS-E&R), Gross Motor Function Measure-88 (GMFM-88) and Functional Independence Measure for children (WeeFIM) were used as outcome measures to assess therapeutic efficacy. Results: 9 months after first transplantation, symptomatic improvements such as reduced spasticity, ability to crawl with reciprocal pattern, independent walking with walker, independent transfer from bed to floor, improved biting and chewing and cognition were observed. On GMFCS-E&R she improved from level III to level II, GMFM-88 improved from 36.36% to 38.62% and WeeFIM improved from 34 to 38. These improvements were well supported by positron emission tomography–computed tomography which showed improved metabolism in bilateral cerebellum and medial temporal cortex. All these improvements were maintained even at 5 months’ follow up post second transplantation. No adverse events were reported after the procedures or at follow ups. Conclusion: Intrathecal administration of autologous bone marrow mononuclear cells are safe and an effective therapeutic strategy in the management of CP.

Introduction

Cerebral palsy (CP) is an umbrella term including a group of permanent disorders of the development of movement and of posture causing activity limitation. It is caused due to non-progressive disturbances occurring in the developing fetal/infant brain [1]. Premature birth and intrapartum asphyxia are the most common causes of CP [2,3]. Oxidative stress that follows hypoxia, ischemia, or infection and glutamate mediated excitotoxicity are said to be important mechanisms of injury to both the white matter and neurons in the developing brain resulting in motor deficits [2]. The motor component of CP is mostly accompanied by disturbances in sensation, perception, cognition, communication, behavior, epilepsy and secondary musculoskeletal problems depending on the region and extent of damage [1]. Although the overall prevalence of CP is approximately 2 per 1000 live births, it is one of the most common causes of serious physical disability occurring in childhood [4].

Medical management of individuals with CP involves physicians offering primary care along with neurological, orthopedic and rehabilitation inputs. Conventional therapies include intramuscular injections of botulinum toxin A, high-protein diets, speech therapy, physical therapy, occupational therapy and orthopedic surgery [5,6]. However, none of these treatment measures can repair the existing damage. Recently, cell transplantation has gained importance as a treatment option for CP owing to its potential of neuroregeneration and neuroprotection demonstrated in the animal brain [7].

To study the efficacy of cell transplantation in CP, we administered cell transplantation to a 2 years and 2 months old female child with CP. Autologous bone marrow mononuclear cells were used for transplantation as their procurement requires a minimally invasive procedure, their safety has been established [8,9] and their use is not ethically restricted [10].
CASE PRESENTATION

Herein, we present a case of a 2.2-year-old female child diagnosed with CP. In the prenatal history, the mother suffered from chickenpox and was on medications for the same in the 8th week of gestation. The child was born at full term through cesarean section and cried immediately after birth. Her birth weight was normal. There was a history of meconium aspiration and breathing difficulty at birth for which she was in the neonatal intensive care for 9 days. The meconium was removed by suction and she was administered oxygen. A two-dimensional echocardiogram was done which revealed patent ductus arteriosus. A repeat two-dimensional echocardiogram echo to be changed to two-dimensional echocardiogram after 2 months revealed ductal closure.

At 9 months of age, parents noticed developmental delay and on consultation with pediatrician she was diagnosed as a case of CP. Magnetic resonance imaging brain was done which revealed bilateral subcortical and deep white matter abnormalities. The acquisition of themilestones is shown in Table 1 [11].

Before administration of bone marrow mononuclear cells, she was examined thoroughly by a team of experts. On examination, Babinski sign was positive with brisk deep tendon reflexes in the lower limbs, while deep tendon reflexes in the upper limbs were normal. There was an increased muscle tone in the plantar flexors, and knee flexors bilaterally. She presented with inability to sit from supine position. Standing balance was poor as she could not stand without support. She could not walk independently and was dependent for ambulation, transfers and bladder bowel hygiene. Her speech was limited to 2-3 words. Biting and chewing were affected and was fed semisolid food only. Cognition was affected as her understanding and concentration were poor. She failed to respond to her name or make eye contact. On the Gross Motor Function Classification System-Expanded & Revised (GMCS-E&R), she was at level III. On Gross Motor Function Measure-88 (GMFM-88) she scored 36.36%. On Functional Independence Measure for Children (WeeFIM), she scored 34.

Magnetic resonance imaging brain revealed diffuse paucity of the bilateral cerebral white matter and mild dilatation of the lateral ventricles. Magnetic resonance imaging-diffusion tensor imaging with fiber tracking was performed. It revealed mild diffuse attenuation of the bilateral cerebral fiber tracts. Brain positron emission tomography–computed tomography (PET-CT) scan revealed severely reduced 18F-fluorodeoxyglucose (FDG) uptake in bilateral cerebellar hemispheres. Mildly reduced FDG uptake was seen in bilateral thalami, hippocampi and amygdala. Rest of the brain parenchyma revealed preserved FDG uptake.

Materials and Methods

Patient selection was based on World Medical Association Helsinki Declaration [12]. Ethical approval was obtained from the Institutional Committee for Stem Cell Research and Therapy. A duly signed informed consent for the procedure was obtained from the patient’s parents.

A detailed neurological examination was performed. Granulocyte colony-stimulating factor (G-CSF, 300 μg) injections were administered subcutaneously. 72 hours and 24 hours before the intervention to enhance the mobilization of bone marrow mononuclear cells [13].

On the day of cell transplantation, 70 ml of bone marrow was aspirated from the right anterior superior iliac spine and collected in heparinized tubes. The mononuclear cells were separated by the density gradient method. The purified mononuclear cells were checked for viability using trypan blue and confirmed using automated TALI machine. The viability of cells was found to be 98%. CD 34+ analysis of these cells was performed using fluorescence activated cell sorting. The total number of cells administered was 9.6×107 and their CD 34+ count was 0.58%. The mononuclear cells were diluted in the patient’s own cerebrospinal fluid owing to its properties of harboring cell growth [14] and were injected intrathecally in the L4-L5 interspace. 200 mg methyl prednisolone in 500 ml Ringer Lactate solution was administered intravenously at the time of transplantation. Following transplantation, the patient underwent a multidisciplinary personalized neurorehabilitation program which included physiotherapy, occupational therapy, special education, speech therapy, psychological therapy, and nutritional advice. A personalized home program was given at discharge and was advised to continue rehabilitation regime at home. She was followed up at 9 months. To enhance the functional improvements seen after the first transplantation, the patient was administered a second transplantation of autologous bone marrow mononuclear cells with the same protocol as described above. The total number of cells administered was 4.20×108 and their CD 34+ count was 1.20%. The purified mononuclear cells were found to have a viability of 97%.

RESULTS

There were no side effects reported immediately after the intervention or at the subsequent follow ups.

9 months post first transplantation, the child could crawl with a reciprocal pattern and walk independently with the help of walker, which she was unable to do earlier. There was decrease in spasticity. She could independently transfer from the bed to the floor. She could perform reach out in all positions. Biting and chewing had also improved and she could eat food items such as a biscuit. On GMFCS-E&R, she moved from level III to level II. GMFM score improved from 36.36% to 38.62%. On WeeFIM she improved from 34 to 38.

Cognitive improvements were observed. Her understanding had improved. She could follow simple commands. She made more eye contact with her parents, and started responding to her name. She could now indicate that she was hungry by crying, making it easier for her parents to understand. Awareness of the surroundings had improved. She started to play more and mingle with her sister. Before intervention she would pass urine and stools in the diaper. But now she would get down from the bed to the floor, indicating to the mother her urge to pass urine/stools. Alertness also improved.
5 months post second transplantation, all the above-mentioned improvements were maintained. In addition, she was now toilet trained and passed urine/stools in potty chair only.

Comparative PET CT performed 9 months post intervention showed improved metabolism in bilateral cerebellum, bilateral thalami and medial temporal cortex including hippocampus and amygdala.

**DISCUSSION**

CP can be divided into subtypes based on tone abnormalities, as spastic, ataxic, or dyskinetic and based on the distribution of limb weakness, as bilateral or unilateral CP [15]. Most cases of CP do not occur due to a single causative factor; but through an interaction of prenatal, perinatal acute or subacute, and postnatal factors that affect brain maturation. These factors contribute to the hypoxic–ischemic brain injury. Hypoxic-ischemia results in a cascade of events including production of proinflammatory cytokines, oxidative stress, deprivation of growth factors, extracellular matrix modification and excessive release of glutamate, causing cell death, predominantly in the white matter in preterm infants and the gray matter in full-term infants [16]. These disturbances result in activation of neutrophils, macrophages and microglia leading to direct or indirect neuronal and preoligodendrocyte cell death or dysfunction [17]. Studies have suggested that the immature white matter can also be damaged by excessive release of glutamates [18]. Gray matter abnormalities in cortical and deep nuclear structures and cerebellar abnormalities may occur and contribute to development of cognitive and psychomotor delays [17]. The treatment of CP is restricted and aimed at increasing functional independence through pharmacotherapy, rehabilitation and use of assistive devices and functional aids [19]. But, these have failed to provide satisfactory benefits. Extensive research has presented stem cell therapy as a treatment option due to their ability to target the underlying pathological mechanisms in CP [20].

In our case, we administered autologous bone marrow mononuclear cells intrathecally. Studies have provided evidence that these cells survive and selectively migrate to injured areas, providing therapeutic benefits in diseases of the central nervous system [21]. Due to avoidance of the risk of immune rejection, autologous cells possess an advantage over allogenic cells [20,22]. Intrathecal route of administration was used to ensure application into the central nervous system and because cerebrospinal fluid possesses properties which support cell growth [14]. Though, the exact mechanism of bone marrow mononuclear cells remains unclear; several mechanisms of action are believed to be involved. The secretion of neurotrophic and growth factors may be the most important mechanism by which bone marrow mononuclear cells

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**Table 1. Gross Motor Developmental History**

<table>
<thead>
<tr>
<th>Domains of development</th>
<th>Milestones</th>
<th>Patient’s age at milestone attainment</th>
<th>Normal child’s expected age at milestone attainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross motor</td>
<td>Head control</td>
<td>10-11 months</td>
<td>1-2 years</td>
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<tr>
<td></td>
<td>Rolling</td>
<td>17-18 months</td>
<td>3-4 years</td>
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<tr>
<td></td>
<td>Sitting with support</td>
<td>20 months</td>
<td>6-8 months</td>
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<tr>
<td></td>
<td>Crawling</td>
<td>Not achieved</td>
<td>8-9 months</td>
</tr>
<tr>
<td></td>
<td>Standing with support</td>
<td>24 months</td>
<td>6-11 months</td>
</tr>
<tr>
<td></td>
<td>Standing independently</td>
<td>Not achieved</td>
<td>12 months</td>
</tr>
<tr>
<td></td>
<td>Crawling</td>
<td>23 months</td>
<td>10 months</td>
</tr>
</tbody>
</table>
produce therapeutic benefits [23,24]. These cells can migrate to the sites of damage and secrete neurotrophins and growth factors, which influence the microenvironment in the damaged area. Neurotrophins and growth factors such as connective tissue growth factor, transforming growth factor beta 1 (TGFβ1), fibroblast growth factors (FGF), vascular endothelial growth factor (VEGF) and nerve growth factor beta (NGFβ) are known to promote neuroprotective and neurorestorative mechanisms in the central nervous system. Neurotrophic factors promote neuronal sprouting, synaptogenesis and increase in neurotransmitter release with increase in neurotransmission [24]. FGF may reduce the size of ischemia and promote migration and proliferation of stem cells [24]. TGFβ1 and VEGF, exhibit neuroprotective effect against ischemia induced apoptosis. Bone marrow mononuclear cells secrete a variety of cytokines which promote hematopoietic cells to adhere to the endothelium promoting angiogenesis [25]. Exosomes which are membrane enclosed vesicles responsible for cell-cell communication are generated by bone marrow mononuclear cells. Exosomes are believed to enhance neurite remodeling, and angiogenesis and may further contribute to functional recovery [26,27]. Administered bone marrow mononuclear cells may also differentiate into oligodendrocytes and replace the damaged/lost ones and promote functional improvement in the affected motor tracts through promotion of remyelination [23,28]. Thus, cell transplantation with bone marrow mononuclear cells possesses the potential to address the core pathogenesis of CP.

Our case study provides evidence of the therapeutic benefits of bone marrow mononuclear cell transplantation. There were no adverse events noted immediately after the procedure or at the subsequent follow-ups. The patient showed improved motor functions and cognitive functions which eventually led to functional improvements. The GMFCS measures gross motor function in children with CP. The GMFCS-E&R reflects the degree of function and independence and as a result improve their quality of life. Cell transplantation can promote acquisition of developmental milestones in CP.

Brain PET CT using FDG was used to monitor disease activity and to determine response to therapy at cellular level. It measures the cerebral metabolic activity, providing the functional status and is also able to measure any alteration in metabolic activity [30]. Increase in FDG uptake compared to pre-intervention was seen in bilateral cerebellum, medial temporal cortex including the hippocampus and amygdala and in bilateral thalami after intervention. Thus, the improvement in motor functions, balance, posture and cognition seen in our patient were well correlated with the changes seen on the PET CT (Refer to Figure 1, Table 2).

PET CT findings supported by functional and motor improvements confirm the therapeutic benefits of bone marrow mononuclear cell transplantation in our case study. However, this is a single case study and hence, may require more evidence to establish its change therapeutic efficacy to therapeutic efficacy.

CONCLUSION
Intrathecal administration of autologous bone marrow mononuclear cells is safe and can be a therapeutic strategy in combination with available conventional treatments for management of CP. To objectively monitor the treatment outcome, PET CT brain can be used as it evaluates the outcome at cellular level with respect to metabolic changes occurring in the brain post intervention. Autologous bone marrow mononuclear cell transplantation in combination with neurorehabilitation helps the CP patients regain their lost functions and make them functionally independent and as a result improve their quality of life. Cell transplantation can promote acquisition of developmental milestones in CP.

REFERENCES


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