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Research Article

Molecular Imaging (PET and SPECT) for Children with Hypoxic-Ischemic-Encephalopathy and Cerebral Palsy Before and After Cell Therapy

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ABSTRACT

Introduction: Glucose metabolism has been the focus of research in order to understand pathological conditions associated with diseases such as neonatal Hypoxic-Ischemic-Encephalopathy (HIE), Cerebral Palsy (CP) and cerebral infarction.

Objective: To evaluate the use of molecular imaging (SPECT and PET) for children with HIE and CP before and after cell therapy, and to propose future perspectives on the use of those modalities for assessment of brain function in children with these conditions.

Methods: PubMed search for studies using PET or SPECT scans for HIE and CP in children.

Results: We identified 18 PET and 17 SPECT studies that have been performed in cases under age of 19 over the past three decades (1991-2021). Six papers on PET use consisted of one with human Umbilical Cord derived Mesenchymal Stromal Cells, one mobilized Peripheral Blood Mononuclear Cells, three autologous Bone Marrow Mononuclear Cells and one allogeneic Umbilical Cord Blood. 4/6 papers reported that PET-CT scan revealed increased glucose metabolism and 1/6 showed no significant change in glucose metabolism after cell therapy. One article on SPECT reported that 2/5 cases had improvement of cerebral perfusion in the thalamus after treatment.

Discussion: SPECT in the first few weeks of life is useful and more sensitive than MRI in predicting major neurological disability. SPECT is not appropriate for neonates because of the risk of radiation, improvement of other clinical test equipment. PET studies reported high glucose metabolisms in the early neonatal period of children with mild to moderate HIE, but not in the most severe cases, including those neonates that died. We suggested that PET could be more useful tool to estimate effectiveness of stem cell therapy than SPECT.

Conclusion: PET might be good clinical modalities to clarify mechanism of stem cell therapy for CP. We need further clinical studies to clarify more precisely.

Keywords: Positron Emission Topography (PET), Single Photon Emission Computed Tomography (SPECT), Cerebral Palsy (CP), Hypoxic-Ischemic Encephalopathy (HIE), Cell therapy

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ABBREVIATIONS

CP: Cerebral Palsy; HIE: Hypoxic-Ischemic Encephalopathy; MSCs: Mesenchymal Stromal Cells; BM: Bone Marrow; BM-MNCs: Bone Marrow Mononuclear Cells; UC-MSCs: Umbilical Cord derived-MSCs; PVL: Periventricular Leukomalacia; UCB: Umbilical Cord Blood; BM-MSCs: BM-derived Mesenchymal Stromal Cells; mPBMCs: mobilized Peripheral Blood Mononuclear Cells; ATP: Adenosine Triphosphate; OHBA: Beta-Hydroxybutyrate; IVH: Intraventricular Hemorrhage; ASD: Autism Spectrum Disorder; IT: Intrathecal; IV: Intravenous; PET: Positron Emission Topography; SPECT: Single Photon Emission Computed Tomography; CT: Computed Tomography; G-CSF: Granulocyte Colony Stimulating Factor; rhEPO: recombinant human Erythropoietin; USG: Ultrasonography; DTI: Diffusion Tensor Imaging

INTRODUCTION

Hypoxic-Ischemic-Encephalopathy (HIE) is one of the most common neonatal conditions and may lead to severe motor difficulties and Cerebral Palsy (CP). Magnetic Resonance Imaging (MRI) studies have been valuable to evaluate the severity of HIE and helped in determining the severity of HIE; (1) mild-involving cortex lesions, (2) moderate-that include the basal ganglia and the thalamus in addition to cortex lesions, and (3) severe-include additional lesions.

We reported in 2018 a correlation between MRI and neurological sequelae in some patients with HIE (Shinomoto T, *et al*., 2018). However, our clinical experience is that there is frequently some noticeable mismatch between MRI findings and neurological sequelae.

Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) studies have been performed to evaluate the extent of HIE and/or CP for several decades, based on the fact that damage to cerebral blood flow and glucose metabolism is one of the most important reasons for HIE and CP (Volpe JJ, *et al*., 1985).

Furthermore, evidence suggest the effectiveness of stem cell therapy for improvement in patients with CP (Nabetani M, *et al*., 2021) that was not associated with paracrine, immune-regulatory, or angiogenesis. Studies of cell therapy for CP reported significant increase of glucose metabolism, as shown on PET studies (Gu J, *et al*., 2020; Sharma A, *et al*., 2015; Min K, *et al*., 2013).

MATERIALS AND METHODS

The purpose of this study was to evaluate the value of molecular imaging (SPECT and PET) for children with HIE and CP before and after cell therapy, based on a literature search from 1991 to 2021 and to propose future perspectives on the use of those modalities for assessment of brain function in children with these conditions.

We conducted a PubMed (MEDLINE) search for studies using PET or SPECT, that included the terms HIE, CP and Encephalopathy, in human subjects under the age of 19 years, in English

and Japanese. We found a total of 18 papers related to the use of PET and 17 to the use of SPECT.

RESULTS

Single Photon Emission Computed Tomography (SPECT)

Six studies included patients with HIE, four reported low cerebral perfusion in the basal ganglia in patients with HIE (Iwaibara T, *et al*., 2010), and low cerebral perfusion in the lentiform nucleus and thalamus in half (3/6) of the patients with severe HIE. Tranquart F, *et al.* reported low corpus striatum-to-cerebellum activity ratio in cerebral perfusion (Tranquart F, *et al*., 2001). Kapucu LO, *et al.* reported low striatal-to-occipital cortex ratio in cerebral perfusion (Kapucu LO, *et al*., 1998) and Oshima M, *et al.* reported low cerebral perfusion in the entire brain (Oshima M, *et al*., 1993). Three studies reported cerebral perfusion of parasagittal lesions (Oshima M, *et al*., 1993; Konishi Y, *et al*., 1994; Shah S, *et al*., 2001). Konishi Y, *et al.* reported low cerebral perfusion of a wide area of the brain, except in the basal ganglia, brain stem and the sensory cortex, in three cases with HIE that suffered severe neurological prognosis, despite no remarkable MRI abnormality (Konishi Y, *et al*., 1994). Oshima M, *et al.* reported low cerebral perfusion in the parasagittal region in cases with mild HIE (Oshima M, *et al*., 1993). Shah reported low cerebral perfusion of parasagittal lesions in 5/12 cases (Shah S, *et al*., 2001) (*Table 1*).

Seven papers included patients with CP, and five reported low cerebral perfusion in the thalamus area. Yamada K, *et al.* reported low cerebral perfusion in the cortex and corpus striatum, in addition to the thalamus (Yamada K, *et al*., 1995). Lee JD, *et al.* reported low cerebral perfusion of the cortex, basal ganglia and cerebellum, in addition to the thalamus (Lee JD, *et al*., 1998). Yim SY, *et al.* reported low cerebral perfusion in the cerebellum in addition to the thalamus (Yim SY, *et al*., 2000). Okumura A, *et al.* reported low cerebral perfusion of the basal ganglia connecting to the cortex (Okumura A, *et al*., 2006). Kao CH, *et al.* reported low cerebral perfusion of occipital lesions in cases with visual disturbances and relevant cortical area in children with spastic quadiplesia (Kao CH, *et al*., 1994). Rana KS, *et al.* has reported low cerebral perfusion of the cortex and a subcortex lesion (Rana KS, *et al*., 2016) (*Table 1*).

Two articles provided information on neonates that were born with Very Low Birth Weight (VLBW). Borch K, *et al.* reported that 13 VLBW cases with Periventricular Leukomalacia (PVL) had low cerebral blood flow in periventricular white matter lesions (Borch K, *et al*., 2010). Valkama AM, *et al.* reported low cerebral blood flow of the cortex, thalamus and cerebellum (Valkama AM, *et al*., 2001) (*Table 1*).

Iivanainen M, *et al.* reported that SPECT was useful for the diagnosis of degenerative brain diseases (82%) (Iivanainen M, *et al*., 1990) and it was more sensitive than Electroencephalogram (EEG), CT and MRI. Konishi Y, *et al.* reported that SPECT is better than other tests if done during the first week of life (Konishi Y, *et al*., 1994). Shah S, *et al.* reported that the relationship between findings in a SPECT exam and neurological sequelae at three months of age had a positive predictive value of 75% (brain Ultrasonography (USG) 60%) and negative predictive value of 100% (USG 76%) (Shah S, *et al*., 2001). Okumura A, *et al.* suggested that SPECT might be useful in cases of kernicterus when no remarkable findings can be demonstrated on an MRI scan (Okumura A, *et al*., 2006).

However, two studies suggested that SPECT might not be the most appropriate test for neonatal HIE because of limited image resolution and risk of exposure to radiation. Indeed, there have been no reports of SPECT studies with neonates since 2016 with one exception-for the study of epilepsy (Konishi Y, *et al*., 1994; Valkama AM, *et al*., 2001).

Of interest, SPECT was useful in one study to demonstrate cerebral perfusion in children with CP after stem cell therapy (Lee YH, *et al*., 2012). Diverse neurological domains improved in five patients (25%) as assessed by developmental evaluation tools as well as by fractional anisotropy values in brain MRI-Diffusion Tensor Imaging (DTI). The neurologic improvement was significant in patients with diplegia or hemiplegia rather than quadriplegia. The procedure was generally well-tolerated, although five patients experienced temporary nausea, hemoglobinuria, or urticaria during the Intravenous (IV) infusion of the autologous Umbilical Cord Blood (UCB) transfusion. They concluded that autologous UCB infusion is safe and feasible, and has yielded potential benefits in children with CP accompanied with improvement cerebral perfusion (Lee YH, *et al*., 2012).

Positron Emission Topography (PET)

A total of 18 studies were reviewed to assess the benefit of PET for assessment of glucose matabolism. Fourteen studies used Fludeoxyglucose (^{18}F) PET, two assessed GABA-A receptor binding using ^{18}F PET (Lee JD, *et al*., 2007; Park HJ, *et al*., 2013) and two other groups reported cerebral blood flow (Kücükali I, *et al*., 1995; Rosenbaum JL, *et al*., 1997) (*Table 2*).

Table 1: SPECT studies on neonatal HIE and CP during 1991-2020

SPECT	Objects	n	Compound	Result
Iivanainen M, *et al*., 1990	Pediatric patients with various neurological diagnosis	60		
Oshima M, *et al*., 1993	HIE	11	^{123}I-IMP	Diffuse ↓, Parasagital ↓
Kao CH, *et al*., 1994	CP, perinatal asphyxia with Mental Retardation (MR) and involved limbs	13	^{99m}Tc-ECD	Motor cortex ↓, Occipital lobe ↓
Konishi Y, *et al*., 1994	HIE, 41-44 post-conceptional weeks	10	^{123}I-IMP	Diffuse without somatosensory ↓, BG ↓, brainstem ↓
Yamada K, *et al*., 1995	CP, ATE	12	^{123}I-IMP	Thalamus ↓, corpus striatum ↓, orbitofrontal ↓, pericentral gyrus areas ↓, prefrontal ↓, medial temporal areas ↓
Sztriha L, *et al*., 1996	CP (7 five with porencephalic cyst), stroke (2), HHES (3), TBI (2)	14	^{99m}Tc-ECD	
Lee JD, *et al*., 1998	CP; SD (35), SQ (11), spastic hemiplegia (2), ATE (2), mixed (1)	51	^{99m}Tc-ECD	Temporal lobe 53% ↓, BG ↓, Thalamus ↓, cerebellum ↓, extra-temporal cortex ↓
Kapucu LO, *et al*., 1998	HIE; mild (6), moderate (10), severe (4)	20	^{123}I-IBZM	ST/OC (Striatal to Occipital cortex) ↓

Yim SY, *et al.*, 2000	CP; bilateral spastic	36	^{99m}Tc-ECD	Thalamus or cerebellar cortex ↓
Valkama AM, *et al.*, 2001	VLBW; birth weight <1,500 g, gestation age <34 weeks	34	^{99m}Tc-ECD	Cerebellar cortex
Tranquart F, *et al.*, 2001	HIE 39.2 w	12	^{123}I-IMP	Striatum/cerebellum activity ratios ↓
Shah S, *et al.*, 2001	HIE; Sanart 2-3	24	^{99m}Tc-ECD	Parasagital ↓
Okumura A, *et al.*, 2006	CP; ATE due to kernicterus	3	SPECT	All hypoperfusion BG related to cortical area ↓
Borch K, *et al.*, 2010	Premature babies; 26-32 W	13	^{99m}Tc-ECD	Periventriclular
Iwaibara T, *et al.*, 2010	HIE; Sanart 2-3	13	^{99m}Tc-ECD	Lentiform nucleus ↓, Thalamus ↓
Lee YH, *et al.*, 2012	CP; SQ (11), hemiplegia (6), SD (3)	20		The neurologic improvement occurred significantly in patients with diplegia or hemiplegia rather than quadriplegia. Autologous CP infusion is safe and feasible, and has yielded potential benefits in children with CP
Rana KS, *et al.*, 2016	CP; Spastic (91%), Asphyxia (69.6%) White matter change including PVL (73.2%)	56	^{99m}Tc-ECD	Cortex↓, sub cortex↓

Note: ↓ Low; CP: Cerebral Palsy; PET: Positron Emission Topography; SPECT: Single Photon Emission Computed Tomography; HIE: Hypoxic-Ischemic Encephalopathy; ATE: Acute Toxic Encephalopathy; ^{123}I-IMP: N-isopropyl-(^{123}I)-p-iodoamphetamine; HHES: Hemiconvulsion-Hemiplegia-Epilepsy Syndrome; TBI: Traumatic Brain Injury; VLBW: Very Low Birth Weight; PVL: Periventricular Leukomalacia; ^{99m}Tc-ECD: ^{99m}Tc Ethyl Cysteinate Dimer; ^{123}I-IBZM: ^{123}I-(S-)-2-hydroxy-3-iodo-6-methoxy-N[(1-ethyl-2-pyrrolidinyl) methyl]-benzamide; BG: Basal Ganglia; SD: Spastic Diplegia; SQ: Spastic Quadriplegia

Table 2: PET studies on neonatal HIE and CP during 1991-2020

PET	Objects	N	Compound	Result
Kerrigan JF, *et al.*, 1991	SQ, SD, hemiplegia, ATE	23	^{18}F-FDG-PET	Cortex ↓ (SD), BG ↓, Thalamus ↓ (ATE)
Suhonen-Polvi H, *et al.*, 1993	HIE	14	^{18}F-FDG-PET	Sensorimotor cortex ↓ cases with delayed development, subcortical lesion ↓, Thalamus ↓, cerebellum ↓, brainstem ↓(neonatal period and 3 mo)
Blennow M, *et al.*, 1995	HIE	6	^{18}F-FDG-PET	Prefrontal Cortex 3/6 ↑, BG 3/6 ↑,
Kücükali I, *et al.*, 1995	SD	3	Germanium68/ Gallium68 PET using ^{15}O	Whole brain
Suhonen-Polvi H, *et al.*, 1995	HIE and hypoglycemia	9	^{18}F-FDG-PET	
Azzarelli B, *et al.*, 1996	HIE	12	^{18}F-FDG-PET	Most severe HIE, BG ↓, Thalamus ↓, brainstem ↓
Rosenbaum JL, *et al.*, 1997	HIE	26	CBF with cesium fluoride scintilation detectors PET	
Wong VC, *et al.*, 2006	CP	4	^{18}F-FDG-PET body acupuncture	Brain glucose metabolism showed a >10% increase in the frontal, parietal, temporal, and occipital cortices and cerebellum after a short course of tongue and body acupuncture
Batista CE, *et al.*, 2007	CP (ATE)	1	^{18}F-FDG-PET	Severe cases, BG ↓, Thalamus ↓, early days after HIE, Transient BG ↑ (ATE)
Lee JD, *et al.*, 2007	SD due to PVL	30	Cerebral GABAr PET by ^{18}F-Fluoroflumazenil	
Park HJ, *et al.*, 2013	hemiplegia (human)	6	^{18}F-Fluoroflumazenil-PET	
Sharma A, *et al.*, 2013	CP and MR	1	PET-CT	Six months following Autologous Bone Marrow derived MNCs therapy, PET-CT scan showed significant increase in metabolic activity in all four lobes, mesial temporal structures and left cerebellar hemisphere, also supported by clinical improvement in IQ, social behavior, speech, balance and daily functioning.

Min K, *et al*., 2013	CP	96	^{18}F-FDG-PET	Compared with the EPO (n=33) and Control (n=32) groups, the pUCB (n=31) group had significantly higher scores on the GMPM and BSID-II Mental and Motor scales at 6 months. ^{18}F-FDG-PET/CT showed differential activation and deactivation patterns between the three groups.
Sharma A, *et al*., 2015	CP	1	PET	
Sharma A, *et al*., 2015	CP	40	PET	Overall, at six months, 95% of patients showed improvements. The study population was further divided into Diplegic, Quadriplegic, and miscellaneous group of cerebral palsy. On statistical analysis, a significant association was established between the symptomatic improvements and cell therapy in Diplegic and Quadriplegic cerebral palsy. PET-CT scan done in 6 patients showed metabolic improvements in areas of the brain correlating to clinical improvements.
Rah WJ, *et al*., 2017	CP	57	^{18}F-FDG-PET	The administration of G-CSF as well as the collection and reinfusion of mPBMCs were safe and tolerable. 42.6% of the patients responded to the treatment with higher neurodevelopmental scores than would normally be expected. Although we observed metabolic changes to the cerebellum, thalamus and cerebral cortex in the ^{18}F-FDG brain PET-CT scans, there were no significant differences in such changes between the mPBMC and placebo.
Fowler EG, *et al*., 2020	Spastic CP	9	^{18}F-FDG-PET	Cortex ↓, cerebellar ↑ in children with less SVMC
Gu J, *et al*., 2020	CP	39	^{18}F-FDG-PET	9 patients received treatments and completed the scheduled assessments. No significant difference was shown between the 2 groups in AE incidence. Additionally, significant improvements in ADL, CFA, and GMFM were observed in the hUC-MSC group compared with the control group. In addition, the standard uptake value of ^{18}F-FDG was markedly increased in 3 out of 5 patients from the hUC-MSC group at 12 months after transplantation.

Note: ↓ Low; ↑ High; ^{18}F-FDG-PET: ^{18}F-Fludeoxyglucose-Positron Emission Topography; CBF: Cerebral Blood Flow; GABAr: Gamma Aminobutyric Acid receptor; PET-CT: Positron Emission Topography-Computed Tomography; BM-MNCs: Bone Marrow derived Mononuclear Cells; EPO: Erythropoietin; pUCB: potentiated Umbilical Cord Blood; GMPM: Gross Motor Performance Measurement; GMFM: Gross Motor Function Measurement BSID-II: Bayley Scales of Infant Development; G-CSF: Granulocyte Colony Stimulating Factor; mPBMCs: mobilized Peripheral Blood Mononuclear Cells; SVMC: Selective Voluntary Motor Control; AE: Adverse Event; ADL: Activities of Daily Living; hUC-MSC: human Umbilical Cord derived Mesenchymal Stromal Cells; CFA: Comprehensive Function Assessment

For those using Fludeoxyglucose (^{18}F) PET, four studies investigated cases with HIE, of whom three reported glucose metabolism of the basal ganglia and the thalamus. Blennow M, *et al.* reported that none of those with low glucose metabolism and half (3/6) of those with high glucose metabolism in the basal ganglia region at two and a half days after birth (Blennow M, *et al*., 1995). Suhonen-Polvi H, *et al.* reported low glucose metabolism in the cortex, basal ganglia and thalamus in cases with neurological sequaela during the first week of life and three months of life. The repeated PET study showed that the uptake of Fludeoxyglucose (FDG) was markedly high and increased in all brain sections of infants with normal development (n=11), whereas those with delayed development (n=4) had significantly lower values ($P \leq 0.005$) (Suhonen-Polvi H, *et al*., 1995). Azzarelli B, *et al.* reported low glucose metabolism of the brain stem region in addition to basal ganglia and thalamus, in severe cases in which 10/12 infants died at the age of 2 to 12 weeks (Azzarelli B, *et al*., 1996).

When it comes to children with CP, 2/3 papers reported cases with spastic diplegia with low glucose metabolism of the cortex (Batista CE, *et al*., 2007; Fowler EG, *et al*., 2020). Two papers reported children with athetoid CP ("dyskinetic Cerebral Palsy") who were found to have low glucose metabolism in the basal ganglia (Batista CE, *et al*., 2007; Kerrigan JF, *et al*., 1991). Batista CE, *et al.* reported that neonates with athetoid CP with transient high glucose metabolism in the basal ganglia (Batista CE, *et al*., 2007). Human Umbilical Cord derived Mesenchymal Stromal Cell (UC-MSC) therapies for individuals with CP showed improvement in motor function and increase in glucose metabolism by PET-CT scan (Gu J, *et al*., 2020).

Wong VC, *et al.* reported that the brain glucose metabolism was more than 10% higher in the frontal, parietal, temporal, and occipital cortices and cerebellum after a short course of tongue and body acupuncture in CP using PET (Wong VC, *et al*., 2006).

Cell therapy

Recently, PET and SPECT have been used for the investigation of the effectiveness of cell therapies. We identified six clinical studies for CP from 18 articles on PET and one from 17 articles who studied on SPECT since 2013 (*Table 3*). PET and SPECT were performed before and after cell therapies for cases with Cerebral Palsy. Six articles on PET consist of one by human Umbilical Cord derived Mesenchymal Stromal Cells (hUC-MSC) (Gu J, *et al*., 2020), one mobilized Peripheral Blood Mononuclear Cells (mPBMCs) (Rah WJ, *et al*., 2017), three autologous Bone Marrow Mononuclear Cells (BM-MNCs) (Sharma A, *et al*., 2015; Sharma A, *et al*., 2013; Sharma A, *et al*., 2015), one allogeneic Umbilical Cord Blood (Min K, *et al*., 2013). Four of six paper reported that PET-CT scan showed much increase of glucose metabolism and one of six no significant change of glucose metabolism after cell therapy. One article on SPECT reported that two from five cases showed improvement of cerebral perfusion in the thalamus by SPECT after autologous cord blood treatment (Lee JD, *et al*., 1998). Most studies were performed using Intrathecal (IT) (n=3) and Intravenous (IV)(n=4) injection. Administration was once in 6 studies and four times in one study. As with adverse events, allogeneic UCB with rhEPO showed ten serious adverse events that required the hospitalization of nine patients among the 105 recruited participants. A 25-month-old female died after allogeneic UCB with rhEPO at 14 weeks post-treatment (*Table 3*).

Table 3: References related to change of PET or SPECT score for neonatal HIE and CP after cell therapy

Reference Number	Disease	N	Route	Cell Type	Cell number	Results	Adverse events
Sharma A, *et al.*, 2013	CP and MR	1	IT	Auto BM-MNC	1 × 1 × 106 CD34+ cells	Six months following Autologous Bone Marrow derived MNCs therapy, PET-CT scan showed significant increase in metabolic activity in all four lobes, mesial temporal structures and left cerebellar hemisphere, also supported by clinical improvement in IQ, social behavior, speech, balance and daily functioning.	None reported
Min K, *et al.*, 2013	CP	96	IV	alloUCB with rhEPO	1 × 3 × 107/kg Total Nucleated Cells (TNCs)	Compared with the EPO (n=33) and Control (n=32) groups, the pUCB (n=31) group had significantly higher scores on the GMPM and BSID-II Mental and Motor scales at 6 months. ^{18}F-FDG-PET/CT showed differential activation and deactivation patterns between the three groups.	Ten serious adverse events that required hospitalization of nine patients were reported among the 105 recruited participants; similar between the three groups. The death of a 25-month-old female in the pUCB group at 14 weeks post-treatment. She was quadriplegic with spasticity from profound hypoxia with involvement of the central gray matter and brainstem. She had severe motor impairment and was unable to control her head. She was medically stable post-treatment with continuous neurological improvement up until the 3-month follow-up evaluation. During routine seizure follow-up, she was found to be neurologically stable. The same day she died during sleep with no apparent cause, and determined not to be related to the treatment.
Sharma A, *et al.*, 2015	CP	1	IT	Auto BM-MNCs	1 × 3.3 × 107 Total Nucleated Cells (TNCs)	On repeating the Functional Independence Measure (FIM), the score increased from 90 to 113. A repeat PET-CT scan of the brain six months after intervention showed progression of the mean standard deviation values towards normalization which correlated to the functional changes. At one year, all clinical improvements have remained.	None reported

Sharma A, *et al*., 2015	CP	40	IT	BM-MNCs	1 × 10.23 × 106 CD34+ cells	Overall, at six months, 95% of patients showed improvements. The study population was further divided into Diplegic, Quadriplegic, and miscellaneous group of cerebral palsy. On statistical analysis, a significant association was established between the symptomatic improvements and cell therapy in Diplegic and Quadriplegic cerebral palsy. PET-CT scan done in 6 patients showed metabolic improvements in areas of the brain correlating to clinical improvements.	At the time of the procedure, there were no complications recorded. During the hospital stay, a few patients did show minor procedure related adverse events-15% a spinal headache, 7.5% nausea, 30% vomiting, 12.5% pain at the site of injection, and 2.5% diarrhea. These events were self-limiting and relieved within one-week using medication. The only major adverse event noted related to cell transplantation was seizures-in 2 patients.
Rah WJ, *et al*., 2017	CP	57	IV	mPBMCs	1st 4.63 ± 2.88 × 108/kg 2nd 6.20 ± 1.94× 108/kg TNCs,	42.6% of the patients responded to the treatment with higher neurodevelopmental scores than would normally be expected. Although we observed metabolic changes to the cerebellum, thalamus and cerebral cortex in the ^{18}F-FDG brain PET-CT scans, there were no significant differences in such changes between the mPBMC and placebo.	Transient hemoglobinuria (n=3) and abdominal pain (n=1) were reported during the mPBMC infusion, and these were resolved with supportive treatments.
Gu J, *et al*., 2020	CP	39	IV	hUC-MSCs	1 × 4.6 ± 0.50 × 107 MSC cells	9 patients received treatments and completed the scheduled assessments. Additionally, significant improvements in ADL, CFA, and GMFM were observed in the hUC-MSC group compared with the control group. In addition, the standard uptake value of ^{18}F-FDG was markedly increased in 3 out of 5 patients from the hUC-MSC group at 12 months after transplantation.	No significant difference between hUC-MSC and control in AE incidence. Serious adverse events were not observed. Upper respiratory infections were reported most frequently (52.6%). Diarrhea (31.6%) fever (36.8%) with a high incidence.
Lee YH, *et al*., 2012	CP	20	IV	Auto UCB	1 × 5.5 ± 3.8 (0.6~15.65) × 107 TNCs	The neurologic improvement occurred significantly in patients with diplegia or hemiplegia rather than quadriplegia. Autologous CB infusion is safe and feasible, and has yielded potential benefits in children with CP.	Infusion was generally well-tolerated, even without premedication, although 3 patients experienced temporary nausea and hemoglobinuria, and 2 patients experienced hemoglobinuria and urticaria, but these were easily controlled with peniramine or intravenous hydration.

Note: CD34+: Cluster of Differentiation 34+; alloUCB: allogeneic Umbilical Cord Blood; rhEPO: recombinant human Erythropoietin; IT: Intrathecal; IV: Intravenous

DISCUSSION

Perinatal complications may result in severe motor disability with a prevalence of 1-2 per 1000 live births in developed countries causing significant burden of illness and necessitating extensive multidisciplinary care (Jacobs SE, *et al*., 2007).

Despite large body of research over the last three decades, no clinically meaningful interventions are offered in order to repair damage to the areas of the brain that were found responsible for control of muscle coordination and movement (Goldstein M, 2004).

Use of SPECT imaging studies among neonates (0-7 days of life) with moderate to severe HIE suggest low cerebral perfusion of the thalamus and basal ganglia regions, despite seeing no such discoveries on MRI scan. Similarly, SPECT findings are associated with low cerebral perfusion of cortex area while none are seen on MRI. Our prior study (Iwaibara T, *et al*., 2010), as well as other published studies, reported that SPECT was a useful modality to identify low cerebral perfusion of the thalamus or orbitofrontal area compared to MRI. SPECT was also shown to be useful to diagnose HIE and CP. Further evidence suggests that SPECT in the first few weeks of life is useful and more sensitive than MRI in predicting major neurological disability. However, because of the risk of radiation, improvements in MR angiography and high cost of SPECT, it is not popular for all neonates. Indeed, there have been no reports of the use of SPECT for evaluation of neonatal diseases such as HIE or CP since 2016, except those evaluating epilepsy. Ultrasonography, MR Spectroscopy or MR angiography, having no risk of exposure to radiation, were the preferred modality (Groenendaal F, *et al*., 2017; Aida N, 2022; Tierradentro-García LO, *et al*., 2021).

Glucose metabolism has been the focus of identifying the pathology associated with cerebral ischemic disease in neonatal HIE, CP and cerebral infarction (Nabetani M, *et al*., 1995). PET studies reported that high glucose metabolisms in the early neonatal periods in children with mild to moderate HIE, but not in the most severe cases, including those neonates that perished. Nonetheless, studies using SPECT reported that cases with severe HIE reported to have low cerebral perfusion. It is possible that the brain may keep sufficient glucose metabolism despite reduced cerebral perfusion. The mechanism of MSCs to improve glucose metabolism might lead to therapeutic potential for individuals CP.

We reported that the importance of glucose for neural activity in hippocampal slices of immature and adult rats during deprivation of oxygen and/or glucose (Nabetani M, *et al*., 1995). We evaluated the relationship between neural activity and energy levels in neonatal brain. During episodes of hypoxia and glucose deprivation, Adenosine Triphosphate (ATP) levels of noted to be preserved in neonates, compared to adult brains. This suggests that energy consumption of the immature brain is smaller than that in the adult brain. During glucose deprivation, neural activity of neonatal rats ceased rapidly although the level of ATP is preserved at high levels. This suggests that glucose plays an important role in the preservation of neural activity in addition to its major function as an energy substrate in neonatal brains (Nabetani M, *et al*., 1995).

Lactate has been shown to be important in maintaining neural function as an energy substrate and energy transporter. We previously reported the possibility of lactate preserving neural function of the adult brain and that glucose metabolites such as lactate and OHBA (Beta-Hydroxybutyrate) are available for both neural activity as well as maintaining the levels of high-energy phosphates in the tissue slice of neonatal rats (Saitoh M, *et al*., 1994; Wada H, *et al*., 1997).

In this study, some article have reported that glucose metabolism improved in clinical experiences for a case with CP after stem cell therapy, evaluated by PET, as well as cerebral perfusion by SPECT. UCB (Umbilical Cord Blood) and peripheral blood mononuclear cells infusion therapy for patients with CP improved brain glucose metabolism. Six months following Autologous BM derived MNCs therapy, PET-CT scan showed significant increase in metabolic activity in all four lobes, mesial temporal structures and left cerebellar hemisphere, also supported by clinical improvement in Intelligence Quotient (IQ), social behavior, speech, balance and daily functioning (Min K, *et al*., 2013). Rah WJ, *et al*. reported that the administration of G-CSF as well as the collection and reinfusion of mPBMCs were safe and tolerable. Close to half (42.6%) of patients responded to the treatment with higher neurodevelopmental scores than would normally be expected. The results showed metabolic changes to the cerebellum, thalamus and cerebral cortex in the ^{18}F-FDG brain PET-CT scans and no significant differences in such changes between the mPBMC and placebo (Rah WJ, *et al*., 2017). Sharma A, *et al*. reported that autologous Bone Marrow Mononuclear Cells therapies for patients with CP also showed improvement of motor function and glucose metabolism. At six months of age, 95% of patients showed improvement. PET-CT scan done in six patients showed metabolic improvements in areas of the brain correlating to clinical improvements (Sharma A, *et al*., 2015). The improvement of glucose metabolism might be caused by improvement of gap junction-mediated cell-cell interaction. In 2020, Kikuchi-Taura A, *et al*. reported that angiogenesis is activated by Bone Marrow Mononuclear Cells *via* gap junction-mediated cell-cell interaction and that cell-cell interaction *via* gap junction is the prominent pathway for activation of angiogenesis at endothelial cells and improvement of glucose uptake. Transplanted BM-MNCs transferred small molecules to endothelial cells *via* gap junction followed by activated Hypoxia-Inducible Factor 1-alpha (HIF-1α) and suppressed autophagy at endothelial cells (Kikuchi-Taura A, *et al*., 2020). We suggested that PET could be more useful tool to estimate effectiveness of stem cell therapy than SPECT (Sztriha L, *et al*., 1996; Suhonen-Polvi H, *et al*., 1993).

CONCLUSION

SPECT in the first few weeks of life is useful and more sensitive than MRI in predicting major neurological disability. SPECT is not appropriate for neonates because of the risk of radiation, improvement of other clinical test equipment. PET studies reported high glucose metabolisms in the early neonatal period of children with mild to moderate HIE, but not in the most severe cases, including those neonates that died. We suggested that PET could be more useful tool to estimate effectiveness of stem cell therapy than SPECT. PET might be good clinical modalities to clarify mechanism of stem cell therapy for CP. We need further clinical studies to clarify more precisely.

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