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RESEARCH ARTICLE

The Role of Autologous Adipose Derived Neural Progenitor Cells with Cognitive and Motoric Function in Cerebral Palsy

Purwati[1, 2, 3*], Asra Al Fauzi[4], Prastiya Indra Gunawan[5], Imam Susilo[2, 6], Diah Puspita Rini[2]

1. *Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia.*
2. *Faculty of Vocational Education, Universitas Airlangga, Surabaya, Indonesia.*
3. *Adjunct Associate Professor, Department of Biotechnology, Asia University, Taichung, Taiwan.*
4. *Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Hospital, Surabaya, Indonesia.*
5. *Department of Paediatrics and Child Health, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo General Hospital, Surabaya, Indonesia.*
6. *Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*

***Correspondence Author: Purwati**

Abstract

Introduction: Cerebral palsy (CP) is primarily a disorder of movement and posture due to neurological damage before, during, or within five years of birth that prevents the brain from developing properly. Supportive treatments, medications, and surgery may conduct, but no optimal results have been obtained. The main goal of this study was to investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post-cerebral palsy patients. Methods: 14 patients were included in this study. Small adipose tissue was isolated by small lipopectomy under local anesthesia, cultured and derived become neural progenitor cells. Intraventricular implantations were performed in the operating room. Patients were evaluated based on the Gross Motor Function Classification System (GMFCS) according to their motor skills before and after treatment in 12 months. The assessment includes cognitive functions, motoric functions and spasticity changes. Descriptive statistics are provided. Results and Discussion: Ten of twelve CP patients (83.33%) had a significant improvement after treatment. The improvement ranged from 1 to 3 levels on the GMFCS score, and improvement was most pronounced in motor skills and cognitive skills. There were no serious adverse events reported, limited to mild headaches, fever or vomiting, and all side effects resolved within few days. Conclusion: Because of the small sample size and non-randomized trial performed, we could not reach a definitive conclusion regarding the potential of intraventricular implantation. However, this small study shows that repeated intraventricular implantation of autologous adipose stem cells is advantageous.

Keywords: *Cerebral palsy, GMFCS, stem cell, Adipose tissue, Neural progenitor cells, Intraventricular implantation.*

Introduction

Cerebral palsy (CP) is primarily a disorder of movement and posture. It occurs when there is neurological damage before, during, or within five years of birth that prevents the brain from developing properly [1, 2]. Prevalence of CP ranging from 1.5 to more than 4 per 1,000 live births or children of a defined age range[3]. About 1 in 323 children has been identified with CP according to Autism and Developmental Disabilities Monitoring (ADDM) [4]. Risk factors include preterm birth, being a twin, certain infections such as toxoplasmosis or rubella and exposure to methylmercury during pregnancy, a difficult delivery, and head trauma in the first few years of life [1]. While movement problems are the central feature of CP, difficulties with thinking, learning, feeling, communication and behavior often occur with 28% having epilepsy, 58% having

difficulties with communication, at least 42% having problems with their vision, and 23–56% having learning disabilities[5,6]. Cerebral palsy is classified based on the type of neuromuscular deficit into (i) spastic (ii) dyskinetic (inclusive of choreoathetoid and dystonic) (iii) ataxic (IV) hypotonic and (v) mixed. Spastic CP is the commonest and accounts for 70%-75% of all cases, dyskinetic – 10% to 15% and ataxic is less than 5% of cases. It is not possible to diagnose CP in infants less than 6 months except in very severe cases. The patterns of various forms of CP emerge gradually with the earliest clues being delay in developmental milestones and abnormal muscle tone [7].

Supportive treatments (include physical, occupational, and speech therapy), medications, and surgery (include lengthening muscles and cutting overly active nerves) may conducted for CP treatment, but no optimal results have been obtained [8]. As the disease progresses, these medications become less effective while at the same time they produce a complication marked by involuntary writhing movement [9].

Surgery to place microelectrodes for deep brain stimulation has been used to reduce motor symptoms, but it is more invasive and full of risks [10]. In the last 10 years, alternative approaches to restoring neural function after cerebral palsy disease have been developed using the concept of neurorestoration using stem cell therapy[11]. Stem cells are multipotent progenitor cells that have been shown to have regenerative as well as imunomodulatory and growth stimulating properties.

They have been shown in vitro to have the capacity to induce angiogenesis and differentiate into different cells types including cells of the nervous system [12]. Stem cell implantation is expected to be a breakthrough in curing CP patients, as it is expected to replace the damaged cells, improve the function of axons, restore the damaged neural circuitry, and induce healing through the activation of endogenous neurogenesis, angiogenesis and synaptogenesis [2,10,12].

Nassim et al reported CP patients treated with intrathecal implantation of bone marrow mononuclear cells had an uneventful post-injection course with 73% of the evaluable patients treated having a good response using the Gross Motor Function Classification System (GMFCS). The average improvement was 1.3 levels with cognitive improvements as well [13]. The GMFCS describes the functional characteristics in five levels, from I to V, level I being the mildest in the following age groups: up to 2 years, 2–4 years, 4-6 years and between 6-12 years. For each level, separate descriptions are provided.

Children in level III require orthoses and assisting mobility devices, while children in level II do not require assisting mobility devices after age 4. Children in level III sit independently, have independent floor mobility, and walk with assisting mobility devices. In level IV, affected children function in supported sitting but independent mobility is very limited.

Children in level V lack independence even in basic antigravity postural control and need power mobility [7]. The main goal of this study was to investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post-cerebral palsy patients. Adipose tissue-derived stem cells are considered to be ideal for application in regenerative medicine, e.g. cerebral palsy.

They can be easily and repeatable harvested using minimally invasive techniques with low morbidity. Adipose tissue-derived stem cells are multipotent and can differentiate into various cell types of the tri-germ lineages, including osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic ß-cells, and hepatocytes. Interestingly, adipose tissue-derived stem cells are characterized by immunosuppressive properties and low immunogenicity. Their secretion of trophic factors enforces the therapeutic and regenerative outcome in a wide range of applications [14].

Material and Methods

Subjects

This study was following the regulatory guidelines of the country. The patients were included if they had confirmed by two neurologists. Prior to the study, informed consent documents, details of the medical treatment and other necessary approval documents were delivered to all patients

after full explanation of the procedure and the safety issues involved. Fourteen patients were included in this study.

The patients were evaluated based on the Gross Motor Function Classification System (GMFCS) [12] according to their motor skills before and after treatment in 12 months. The assessment include cognitive functions (all ways of communicating including speech, gesture, facial expression and augmentative, alternative communication), motoric functions (looks at movements such as sitting, walking and use of mobility devices), and spasticity changes. The assessment was done with the care taker and primary care physician. Descriptive statistics are provided. The following inclusion and exclusion criteria were used for the patient (Table 1).

Table 1: Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
- Cerebral palsy patients - Aged minimum 6 months - Gross Motor Function Classification System (GMFCS) Severe: 4-5 Mild: 1-3	- Significant cardiac, renal or hepatic impaitments - Have encephalitis or meningitis - Have active infection/disease

Table 2: Data progress of post-treatment patients

No.	Gender (M/F)	Age (years old)	48-Weeks Evaluation		Improvement
			Pre-GMFCS*	Post-GMFCS*	
1.	M	7	4	4	• Cognitive functions improved → understanding of spoken sentences and many instructions • No motoric and spasicity changes
2.	M	5	3	2	• Cognitive functions improved → understanding many instructions • Motoric functions improved → head control and balance walking • Spasicity improved
3.	M	8	3	2	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control and balance walking • No spasicity changes
4.	M	5	4	3	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control and balance walking • No spasicity changes
5.	M	4	4	3	• Cognitive functions improved → vocabulary used, understanding of spoken sentences concentration improved • Motoric functions improved → head control and balance walking No spasicity changes
6.	F	1	4	3	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control and balance walking • No spasicity changes
7.	M	9	4	2	• Cognitive functions improved → vocabulary used, understanding of spoken sentences and many instructions • Motoric functions improved → head control, balance walking and jumping • Spasicity improved
8.	M	9	4	4	• Cognitive functions improved, but not significant

					• No motoric and spasicity changes
9.	M	2	3	2	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control and balance walking • No spasicity changes
10.	F	11	4	1	• Cognitive functions improved → vocabulary used, understanding of spoken sentences and many instructions, more talk and cheerful, can express her whishes, concentration improved • Motoric functions improved → head control, balance walking and jumping • Significant spasicity improvement
11.	M	3	4	2	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control, balance walking • Spasicity improved
12.	F	4	3	2	• Cognitive functions improved → vocabulary used, understanding of spoken sentences • Motoric functions improved → head control, balance walking • No spasicity changes

* GMFCS Score:
Level 1 = perform running and jumping, but speed, balance and coordination are limited
Level 2 = may walk with physical assistance, a handheld mobility device or used wheeled mobility
Level 3 = use wheeled mobility when traveling long distances and may self-propel for shorter distances
Level 4 = require physical assistance or powered mobility in most settings
Level 5 = transported in a manual wheelchair in all settings

Abbreviations: CP, cerebral palsy; GMFCS, Gross Motor Function Classification System; ADDM, Autism and Developmental Disabilities Monitoring; SVZ, sub ventricular zone

Procedure and Implantation Techniques

Isolation and intraventricular implantation of adipose derived neural progenitor stem cells were performed in the operating room. Autologous adipose tissue isolation was performed under local anaesthesia, and aspiration was performed with a sterile procedure. Neural progenitor cells were derived from autologous adipose tissue. Small adipose tissue was isolated by small lipopectomy under local anesthesia after isolation then cultured and derived become neural progenitor cells for around 3 weeks. Before used, neural progenitor cells was validated.

Characterization of neural progenitor cells by expression of L-Dopa with immunocytochemistry and expression of Notch using flow cytometry (NPCP technique by Purwati). Under general anaesthesia, patients were conditioned in a supine position. The hair was shaved just behind the right frontal hairline, and the area was washed with antiseptic solution. A mark was made on the right Kocher point. A 2.5-cm wide linear incision was made in layers through the periosteum. The process was continued by creating a burr hole in the calvaria and a small dural incision. An Ommaya reservoir was inserted into the ventricle, and then a maximum of 5 cc cerebrospinal fluid was slowly aspirated through the Ommaya reservoir with a wing needle.

Neural progenitor cells were transplanted with the same wing needle (2 x 10^6 cells in 3cc normal saline) and flushed with 2cc normal saline. The surgical wound was then sutured layer by layer. For booster implantation, the same procedures were performed without the open procedure or general anaesthesia one month after the first implantation. Hair did not need to be shaved, disinfection with povidone-iodine was performed at the skin and stem cell injection was carried out with the same dose using wing needle no. 25 through the subcutaneous transplanted Ommaya reservoir. Booster implantation was done twice at one-month intervals.

Results

This study obtained 9 patients (75%) were male, and 3 patients (25%) were female. The youngest patient was 1 years old, and the oldest was 11 years old. There were no

serious adverse events reported, limited to mild headaches, fever or vomiting, and all side effects resolved within few days. Ten of twelve patients had a significant improvement after stem cell therapy (83.33%). The improvement ranged from 1 to 3 levels on the GMFCS scoring system. The improvement was most pronounced in motor skills and cognitive skills in 10 patients (83.33%) and in many cases it was the first feature noted to improve. Although not everypatient improved, overall, the majority of patients showed significant improvements. Further details on patient characteristics improvements are shown in Table 2.

Discussion

Stem cells, including adipose tissue-derived stem cells, have emerged as a key element of regenerative medicine therapies due to their ability to differentiate into a variety of different cell lineages. Their capacity of paracrine secretion of a broad selection of cytokines, chemokines, and growth factors make them highly clinically attractive. Adipose tissue-derived stem cells have been shown to have the capacity as anti-apoptotic, anti-inflammatory, immunomodulatory, anti-scarring effects and proangiogenic, which make these cells promising candidates for cellular therapy in regenerative medicine [14, 15]. Brain is control center of the body.

This organ has a wide range of responsibilities from coordinating our movement to manage on emotion. For almost hundred years, many studies show that brain cell do not regenerate so need to add new neuron when the brain injured. In this study, the source of neural progenitor cells we used from autologous adipose tissue by small lipopectomy, because neural progenitor cells high expressed from adipose derived compared with from bone marrow derived, with expression of Notch and L-Dopa [16, 17].

There is no standardized dose for stem cell therapy associated with the route of administration and the type of disease. For example, an overly high dose in intraparenchymal implantation can affect the nutrition of grafted cells and, if given intravascularly, cause micro-emboli and vessel occlusion [18]. In this study, we used the dose of 2 x 107 stem cells with the intraventricular route applied directly into the intracranial space. This route makes the dose adjustment is more flexible, because it can be controlled by reducing the ventricular fluid if necessary based on the transplant dose. The risk of increased intracranial pressure and mass effects of the body can also be avoided.

This dose was administered in 3 ml of fluid to avoid highly concentrated doses and excess fluid volume. No complications, such as signs of increased intracranial pressure, infections or seizures were observed. The ventricular system has thin walls composed of ependymal cells. The permeable properties of ependymal cells make it quite effective for the treatment of certain medicines, including stem cell therapy targeting the brain parenchyma [19, 20]. On the lateral ventricle, the ventricular walls are surrounded by the sub ventricular zone (SVZ), which continuously produces new neurons [6].

The location of the neurogenic niche area is very close to the lateral ventricle, which explains why the administration of stem cells through the intraventricular route is an effective method for stem cell therapy in this study. The lateral ventricles are easy to access, enabling direct stimulation of the SVZ. Moreover, cerebrospinal fluid is the endogenous regulatory factor of neuronal differentiation in neural regeneration, where the plexus choroideus produces substances during brain development or the regeneration process after brain injury [21].

The results in all subjects showed no decrease in neurological status and no complications associated with the actions and effects from stem cells. Some possible side effects that could be observed after treatment are increased intracranial pressure, seizures, infection and rejection reaction by the body. However, this study demonstrated that this technique is safe and reported no complications. One other advantage, the presence of the reservoir, facilitates repeated injections when applying booster therapy.

The Gross Motor Function Classification System (GMFCS) looks at movements such as sitting, walking and use of mobility devices in patients with cerebral palsy. It is helpful because it provides families and clinicians with a clear description of a child's current motor function, and an idea of what equipment or mobility aids a child may need in the future. GMFCS uses 5 levels describing the motor function limitations and taking into consideration age, the use of

mobility aids and the quality of movement [22]. Levels 1 to 5 of the GMFCS describe worse dysfunctions and less dependence during mobility as the level goes up.

Levels 1 and 2 have almost independent mobility, while level 3 can move with assistive devices, and levels 4 and 5 are significantly limited and dependent on their helpers for minor movements. There are several effective mechanisms of action involved, including neural cells regeneration, neurons direct stimulation, and trophic paracrine mediators. There is evidence those growth factors like stem cell may help improve brain regeneration [23].

Adipose tissue may generate neurons and other supportive cells. Transplanted adipose-derived neural progenitor cells infiltrate the brain and may help regenerate new elements or combat the neurodegenerative process, fibrosis, and oxidative insults. Neuroprotection may involve release of several neurotrophic factors that work through paracrine and/or-autocrine interactions [13].

Sharma et al reported 85% improvement among cerebral palsy cases with stem cell therapy, where 75% reported improvement in muscle tone and 50% in speech among other symptoms. No significant adverse events were observed [24]. In this study, there was an amazing improvement in neurological status and the quality of life in almost all patients.

Conclusion

In this study, patients with cerebral palsy conducted using GMFCS after 48 weeks of treatment using adipose-derived neural progenitor cells showed an amazing improvement. There were no serious adverse events reported, limited to mild headaches, fever or vomiting, and all side effects resolved within few days. Because of the small sample size and non-randomised trial performed in this study, we could not reach a definitive conclusion regarding the potential of intraventricular implantation. However, this small study shows that repeated intraventricular implantation of autologous adipose stem cells is advantageous.

Stem cell-based therapies are performing more experimental researches as well clinical trials are crucial for advancement of knowledge.

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Disclosure

The author reports no conflicts of interest in this work.

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