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Short Communication

Wharton's Jelly Derived Mesenchymal Stromal Cells and Secretome in the treatment of Autism: an observational Case Report

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Abstract

Autism spectrum disorder (ASD), is a set of heterogenous neurodevelopmental disorder strongly linked with genetic and environmental factors and characterized by atypical patterns of repetitive verbal and nonverbal behaviours including dysfunction in social interaction and communication skills .Though remain unclear , recent evidence has suggested that immune dysregulation as well as neuroinflammation has an a etiological role. In particular, elevation of pro-inflammatory cytokines and chemokines as well as cytokine imbalances in CSF and blood are associated with behavioural and cognitive impairment and dysfunction of immune cells. The current treatment approach consisting of behavioral therapies, pharmacological and nutritional supplementation is at best providing symptomatic relieve. Given the steady rise of ASD over the past decade, there is an urgent need to provide effective therapeutic solutions.

Mesenchymal cells well recognised for its immunomodulatory capabilities has the ability to reduce the impact of inflammation and tissue damage. We present here a case of Autism in a 5 year old female who following two intravenous implantation of Wharton's jelly derived mesenchymal stromal cells (WJ-MSC transplantation) and 4 intramuscular secretome demonstrated improvement in eye coordination, verbal communication, gross and fine motor skills, social skills and emotional regulation. The therapeutic effects of MSC is likely attributed to cell mediated modulation, molecular mediated neuroprotection and restoration of functional neurologic circuitry. There has been no adverse reaction reported during the one year follow-up. Hence it is reasonable to consider WJ-MSC and secretome as an adjunct therapy to conventional approaches in the management of ASD.

INTRODUCTION

The number of diagnoses for autism spectrum disorder (ASD) in Malaysia have risen steadily over the past decade, according to Ministry of Health (MOH). An MOH study in 2005, which used the Modified Checklist for Autism in Toddlers (M-CHAT) screen for ASD, estimated that the prevalence of ASD in Malaysia is between one and two per 1,000 among children aged 18 months to three years. The study also found that male children are four times more likely to get ASD than female children. For the 10-year period from 2010 until 2021, about 19 per cent of children aged 18 and below were diagnosed with ASD compared to other types of learning disabilities.

The actual cause of autism is unclear; however, it has been suggested that the development of autism may stem from the interplay between genetic, biological and environmental factors [1]. The onset of autism usually takes place in early childhood, characterised by impairment in social interaction, verbal communication, repetitive behaviour, restricted motor coordination and reduced cognitive ability [2]. Elevation of pro-inflammatory cytokines and chemokines in CSF and blood are associated with behavioural and cognitive impairment in autism. Furthermore, cytokine imbalance contributes to the alteration of immune cell function [3].

Patients with autism usually undergo neurorehabilitation program such as behavioural therapy, speech therapy and occupational therapy to address behavioural symptoms and promote well- being. Besides that, prescribed medication is given to help ameliorate other comorbidities.

Allogeneic MSC has generated positive effects on patient's social behaviour, motor coordination, emotional regulation and well-being [1,4,5]. We report our observation in using a non-conventional approach utilizing both mesenchymal stem cell (MSC) as well as its secretome in a 5 year old girl with ASD.

MATERIALS AND METHODS

Isolation of primary cell culture from Wharton's Jelly

Umbilical cord was collected from healthy donor after

Caesarean delivery and immediately transferred to the laboratory after mother's consent. Wharton's Jelly mesenchymal stem cells (WJ—MSCs) were isolated from WJ of umbilical cord and cultured and expanded to passage 4 (P4) at 37°C and 5% CO2 in complete culture medium at 95% relative humidity.

Briefly, the umbilical cord was washed with washing buffer and disinfected by soaking in 70% IPA. The umbilical cord was cut into 2 to 3 cm small pieces and each piece was cut open lengthwise followed by removal of umbilical blood vessels (vein and arteries). The umbilical cord was treated with digestion solution, incubated at 37°C for 14 ± 2 hours. After incubation, complete culture medium was mixed with digested cord and centrifuged. The jelly mixture was collected and seeded in complete culture medium in T75 culture flasks and incubated at 37°C, 5% CO2 and 95% relative humidity.

WJ-MSCs were cultured and expanded to passage 4 (P4) and cryopreserved. All cells preparation, cultivation and cryopreservation procedures were conducted by Beike 23 Century International Stem Cell Laboratory, an MOH cGMP/cGTP accredited facility.

Preparation of human umbilical cord mesenchymal stem cells conditioned media (Secretome)

The cryopreserved P4 cells was thawed, resuspended in complete culture medium and centrifuged at 300 x g. The cells pellet was resuspended and seeded in complete culture medium and incubated at 37°C, 5% CO2 and 95% relative humidity for expansion until 75% to 85% cell confluency. Cultured cells were harvested by removing supernatant from cultured flask and poured into a sterile T75 flask for further use. Then, the cells were rinsed with saline and treated with dissociation buffer to detach cells from the flask. The dissociation process was neutralized using collected supernatant. The cell suspension was collected and centrifuged at 300 x g for 10 minutes. After removing the supernatant, the cell pellet was resuspended in complete culture medium. Then the resuspended cells were seeded into sterile culture flask, and incubated at 37°C, 5% CO2 and 95% relative humidity.

After cultured cells reached 75% - 80% confluency, the supernatant was discarded and attached cells were rinsed twice with washing buffer. Then, 3% of issuance buffer was added and cells were incubated for 18 hours. After incubation, the MSC conditioned media were collected and filtered using a 1L filter unit system with a membrane pore size of 0.22µm. Subsequently the conditioned media was diluted with saline and dispensed into vials until further use.

Quality control analyses were carried for the following: mycoplasma analysis was determined according to. Ph. Eur. 2.6.7 using direct culture method and PCR; bacterial endotoxin analysis was determined according to Ph. Eur. 2.6.14. Using gel clot limit test. Microbial limit testing according to US-61 using plate count method; and Sterility testing according to USP-71 using automated microbial detection system.

Therapy

The therapy consists of two doses of Wharton's Jelly MSC (WJ-MSC) administered intravenously, each dose containing 25 million cells/ml, given six months apart followed by four intramuscular injections of secretome.

RESULTS

In this case study, we observe the outcome of receiving WJ-MSC and secretome in addition to rehabilitation therapy in a female, aged 5 with confirmed diagnosis of autism spectrum disorder.

Prior to receiving MSC treatment, patient has undergone rehabilitation therapy comprising of occupational therapy, speech therapy and special education to improve behavioural symptoms. She presented sensory processing issues and difficulty in balancing and movement when first diagnosed. Besides that, she also displayed limited interaction and communication with others. After the initial assessment, she was referred to speech and occupational therapists for rehabilitation sessions. Minor improvements of emotional regulation, gross and fine motor skills, social interaction and communication skills were observed behavioural symptoms such as episodes of meltdown, self-stimulation, sensitive to sounds, afraid of new people and environment and lack of concentration are still prominent.

Following WJ-MSC implantation and intramuscular secretome, patient demonstrated further improvement in different areas such as eye coordination and verbal communication, gross and fine motor skills, social skills and emotional regulation. During and after each treatment, patient was closely monitored for any serious adverse events. No serious adverse effects were reported after each administration of WJ-MSC and secretome.

Individualized Education Plan (IEP) tailored to her condition was used to evaluate her progress in learning and development as well as to provide training throughout the period of receiving therapies. Positive feedback was given by therapists and teachers, commenting that notable and consistent progress has been made (Table 1). Patient was able to conduct daily activities such as throw rubbish with more ease. Patient was able to articulate her feelings through emotions board. Moreover, patient shows longer attention span when performing tasks without displaying stereotypic behaviour. Frequency of inappropriate speech and hyperactivity have also diminished.

DISCUSSION

The global incidence rate of autism is 1 in 100 children as reported by WHO in March 2022 [6,7]. Common pathophysiology observed in patients include neuronal inflammation, brain hypoperfusion and gastrointestinal issues. Inadequate blood supply to the brain affects several processes essential for normal brain function and development [8]. Disruption of neuronal differentiation, outgrowth of axon and dendrites and synaptic dysregulation contribute to poor cognitive abilities observed in

Table 1: Summary of major improvements made during the course of training program.

Key indicators	Before WJ-MSC therapy	After WJ-MSC and Secretome therapy
Social skills	Limited social interaction Refuse to join circle time	Improved social interaction and verbal expression
		Engage with classmates and teachers (greeting and during playtime)
		Able to identify her friends Improve social awareness
Verbal communication	Only use single words to communicate	Able to use more than one word to communicate
Gross motor and balance	Difficulty in balancing body or walking on uneven surfaces	Able to balance herself and walk with minimal support
	Need support most of the time	
Fine motor skills	Difficulty in matching puzzle with missing pieces	Able to perform more complex tasks such as crumple and tear paper, cutting snips with some guidance
	Able to perform simple tasks such as unscrew bottle cap and pick up marble	
Life skills	Able to perform daily activities	Able to complete tasks with minimal guidance
		Keep her own belongings into the bag
Cognitive skills	Understand simple questions	Comprehend one step commands
	Recognise pictures on flashcards	Able to identify new words (adjectives)
		Respond to questions with visual aids
		Able to name her favourite occupation
Emotional behaviour	Easily lose focus with inappropriate speech when carrying out tasks	Able to adapt to new environment and people
	Afraid of new environment and people	Improvement in managing emotions (able to self- regulate)
	Sensitive to sounds	Able to express and share emotions through feelings board
	Meltdown behaviours Stereotypic behaviour	

autism. Apart from that, immune dysfunction is also apparent in patients with autism.

Mesenchymal Stromal Cells (MSC) are multipotent cells with self-renewal properties, capable of stimulating regenerative and healing properties of damaged tissues via paracrine effects [1]. Neuroprotective and immunomodulatory effects make MSC a promising candidate for targeting inflammation and neuronal impairment in autism. A preclinical study showed behavioural improvement, increased BDNF levels and increased neurogenesis in MSC treated mice [9]. Clinical studies have demonstrated safety and efficacy of using umbilical cord derived MSC (UC-MSC) in treating autism [1,10,11]. No observable adverse events were reported following UC-MSC transplantation in these studies and patients have shown improvement in social interaction and verbal communication after receiving UC-MSC therapy. Behavioural symptoms have also significantly reduced.

Several benefits of using WJ-MSC include low immunogenicity, most likely non-tumorigenic, lack of ethical issues, non-invasive and easy collection, high proliferative capacity and easily obtained [12]. MRI scans of autistic brains have shown structural differences and abnormal connectivity in critical areas associated with social deficit and repetitive behaviour [13]. Active neuroinflammation and elevated production of pro-inflammatory cytokines (IL-1β, IL-8 and IL-12p40) were found to contribute in long term immune alteration in autism [14].

Patients with autism usually undergo neurorehabilitation program such as behavioural therapy, speech therapy and occupational therapy to address behavioural symptoms and promote well- being. Besides that, prescribed medication is given to help ameliorate other comorbidities. MSCs offers a unique approach to restore functions which have been lost due to neuropathological processes. MSCs have the capacity to repair and restore damaged neuronal function through its neuroprotective properties [5,15]. Transplanted MSC was able to rescue cerebellar Purkinje cells in animal models by promoting synaptic plasticity and functional recovery [16]. Apart from that, MSC is capable of resetting immunoregulation through the secretion of growth factors, anti-inflammatory cytokines and mediators [13]. Immunomodulatory effects of MSC could supress the proliferation of different immune cell types, reduce the release of pro-inflammatory markers and further inflammation. Another key effect of MSC is the ability to promote angiogenesis which improves vascular perfusion to the brain by the secretion of angiogenic factors [13].

Our observation of this single case of ASD has demonstrated that WJ-MSC and secretome therapy has been well tolerated with no adverse event reported after each administration. It has in fact generated positive effects on patient's social behaviour, motor coordination, emotional regulation and well-being.

CONCLUSION

Proper neurodevelopment at early age is crucial for optimum brain functioning. In the case of autism, there is insufficient vascular blood flow causing brain tissue hypoxia and increase apoptosis, resulting in underdeveloped brain tissues and neurological delays which later manifest into clinical symptoms observed in individuals with autism [8].

In our case study, patient had exhibited apparent developmental delays prior to receiving intervention. Our treatment regime using WJ-MSC and its secretome has helped patient achieve a developmental milestone from inconsistent behavioural patterns to making consistent progress. WJ-MSC therapy has generated positive effects on our patient's social behaviour, motor coordination, emotional regulation and well-being. The use of WJ-MSC combined with other therapies will likely result in synergistic effects reducing the degree of

impairment hence improving patient's quality of life [17]. Long term observation is needed to evaluate the sustainability of combination therapy and whether repeated doses of WJ-MSC and secretome will be necessary.

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