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Cell-Based Therapy for Cerebral Palsy: A Puzzle in Progress

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Abstract

Cell-based therapy has shown promising outcomes in the treatment of cerebral palsy (CP). However, there is no consensus on a standard therapeutic protocol regarding the source of cells, optimal cell dose, timing and frequency of cell injections, route of administration, or the use of combination therapy. This lack of consensus necessitates a comprehensive investigation to clarify these crucial yet undefined factors in cell-based therapy for CP patients. In this commentary, we discuss and compare the trends in Gross Motor Function Measure-66 following intrathecal injection of umbilical cord blood mononuclear cells (UCB-MNCs) and umbilical cord tissue mesenchymal stromal cells (UCT-MSCs) in children with CP. Our study revealed that MNC injections led to earlier improvements in gross motor function, whereas MSC applications resulted in more sustainable changes. These findings provide key insights into the efficacy of different cell types, which will be beneficial for future studies and for refining cell-based therapy protocols for CP treatment.

Keywords: Cerebral Palsy, Mesenchymal Stromal Cells, Mononuclear Cells, Umbilical Cord Blood

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Cerebral palsy (CP) is the most common neurologic-based physical disability in children. This lifelong disorder is a result of non-progressive brain injuries compatible with CP criteria, including white matter lesions that may occur in the perinatal period due to hypoxia/ischemia encephalopathy and intraventricular/ intraparenchymal hemorrhage (1). Motor function impairment is one of the prevalent problems of children diagnosed with CP, and it should be addressed efficiently in the design of novel treatment protocols (2).

Cell-based therapies as novel treatment approaches for CP patients were launched 15 years ago (3-6); however, the final outcomes of these studies were controversial, and fragmented, and the results are inconsistent across studies. This could be because of heterogeneity in the pathophysiology of CP and the different clinical settings for each trial (7).

Based on many studies, applying umbilical cord blood mononuclear cells (UCB-MNCs) and umbilical cord tissue mesenchymal stromal cells (UCT-MSCs) are safe and effective in neurological disorders (8-10). However, the effects of various cells, different doses, frequency of injections, suitable interval between the injections, and local versus systemic administration of cells in a defined period have not been compared, and the beneficial impact of each variable has not been scored. In this study, we aimed to compare the effect of two different cell types on the improvement of gross motor function following UCB-MNCs and UCT-MSCs intrathecal injection over a one-year follow-up.

Our research group has recently conducted two cell therapy-based clinical studies with the same design but

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two different cell types in which 108 participants out of 391screened cases with spastic CP aged between 4 to 14 years, Gross Motor Function Classification System (GMFCS) between 2 and 5, and white matter lesions compatible with CP criteria were eligible and recruited. They were randomly and equally allocated into three groups including one control and two intervention groups. The participants in the intervention groups received a single intrathecal (IT) injection of either 5×10^6/kg UCB-MNCs or 20×10^6 UCT-MSCs and were followed for a year according to the timeline illustrated in Figure 1.

The primary outcomes for both studies were safety and clinical improvements in the Gross Motor Function Measure-66 (GMFM-66), Quality of life (CP QoL), Pediatric Evaluation of Disability Inventory (PEDI), and the Manual Ability Classification System (MACS) scores at 1, 3, 6, and 12 months after the intervention. Secondary outcomes were any improvement in magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and diffusion tensor imaging (DTI) at baseline and 12 months after cell injection (Fig.1). We compared the effect of two different cell sources and the control group based on the GMFM-66 which has validity and sensitivity for assessing motor function of children with GMFM-66 (11).

The results of these two clinical studies were pooled for this comparative study. The statistical analyses were carried out using IBM SPSS Software (IBM, USA, version 25.0) and GraphPad Prism (GraphPad Software, Inc., USA, version 7.04) by a blinded statistician. Two-sided significance testing was performed, and $P<0.05$ were considered statistically significant. To measure effect sizes, Cohen's d test with a 95% confidence interval (CI) was utilized. Effect sizes were categorized as small (d 0 to 0.20), medium (d 0.20 to 0.50), or large (d > 0.50) using the R statistical package (R Core Team, 2013). Detailed protocol of analysis is described before. Intention-to-treat (ITT) analysis was performed for all participants that were included in the statistical analysis (12).

Thirty-six participants were allocated to each group with a mean age of 112.5 (36.6) months, 101.7 (32.1) months, and 102.5 (29.9) months, and the proportion of male participants were 69.4, 58.3, and 52.8% in UCB-MNC, UCT-MSC, and control groups, respectively (Table 1). The results related to the safety and efficacy of one IT injection of UCB- MNCs (13) and UCT-MSCs in CP patients (12) were published in two original articles which indicate the interventions significantly improved motor function, reduced spasticity, and resulted in a better quality of life in CP patients.

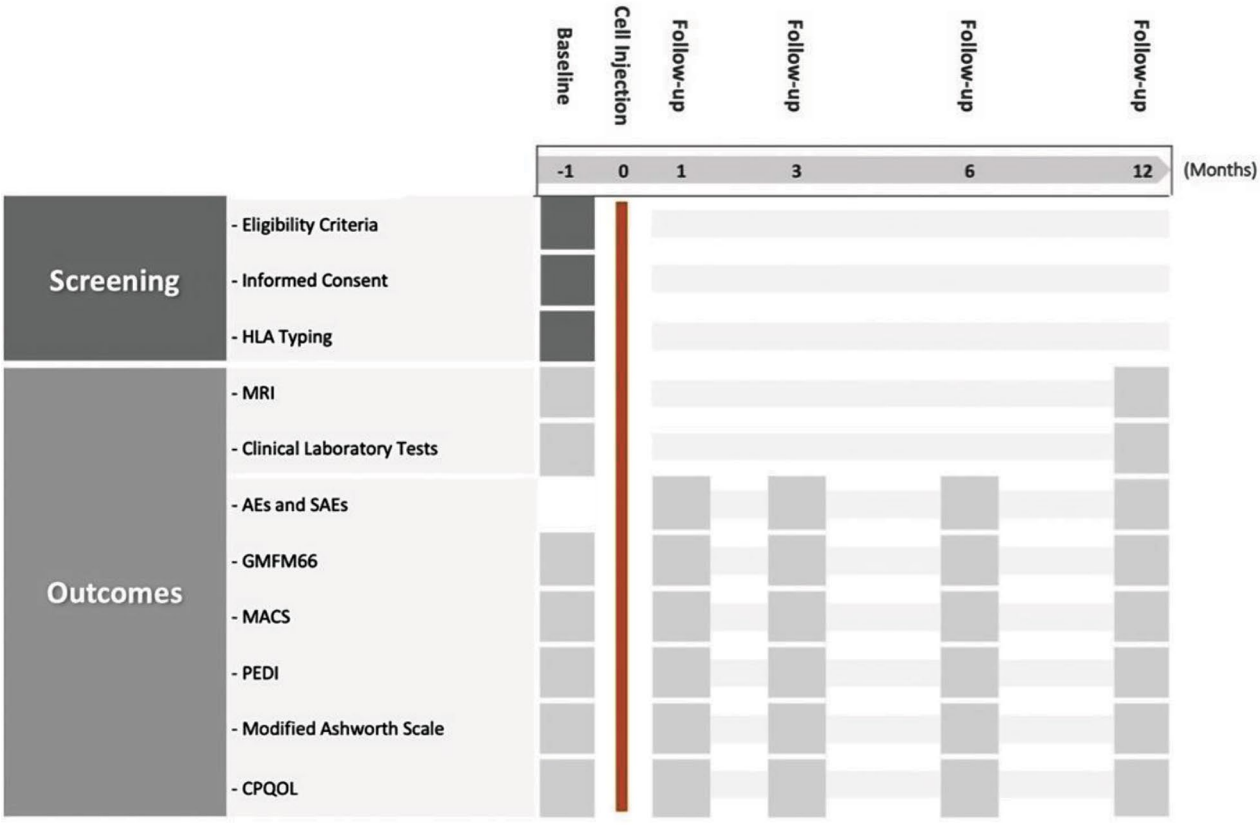

Fig.1: The timeline of the study. The patients received one cell transplantation and completed four scheduled visits at 1 month (± 7 days), 3 months (± 7 days), 6 months (± 7 days), and 12 months (± 15 days) after intervention.

A relative comparison of the results of both studies revealed that significant improvements in gross motor function based on the GMFM-66 scale were observed in both intervention groups with mean changes from the baseline of 9.24 (2.87), 4.47 (5.21) and 0.9 (3.88) in UCB-MNC, UCT-MSC and control groups, respectively, at the first visit one month after the cell injection (Fig.2). The GMFM-66 score was significantly higher in the UCB-MNCs group than in the UCT-MSCs group in the first month after the intervention ($P<0.05$). This suggests that the application of UCB-MNCs resulted in a steeper slope in improving motor neuron function. This improvement may be related to the paracrine effect of the cells and their heterogeneity, leading to facilitated angiogenesis and neurogenesis. This pattern is maintained up to the 3rd month after intervention.

The trends of GMFM-66 changes in both groups of interventions continued improving up to the 6th month after intervention. However, the improvement trend of the UCT-MSCs group showed better results between the 1st and the 3rd months post-injection. This may be because of their proliferation capacity, their plasticity, the production of trophic factors, and their paracrine effect (14, 15).

Six months after the intervention, gradual deterioration of the improvements was observed (Fig.2). The mean changes from the baseline reduced from 11.26 at the six-month follow-up to 9.62 at the 12-month follow-up in the MNC group and, respectively, from 11.27 to 10.65 in the MSC group. However, the mean changes remained significant in the intervention groups in comparison with the control group ($P<0.05$). This could be due to the senescence or inactivation of transplanted cells (16).

Based on these repeated injections with shorter intervals could be proposed to maintain, extend, and enhance the desired efficacy of the transplanted cells as other studies have suggested (17, 18).

Table 1: Demographic characteristics of participants

Demographics	Control (n=36)	MNC (n=36)	MSC (n=36)	P value
Sex, n (%)				
Female	17 (47.2)	11 (30.6)	15 (41.7)	0.213
Male	19 (52.8)	25 (69.4)	21 (58.3)	
Age, months				
Mean (SD)	102.5 (29.9)	112.5 (36.6)	101.7 (32.1)	0.422
Median (rang)	96 (62-156)	96 (60-196)	96 (60-192)	
Age ranges, n (%)				
<100 months	18 (52.9)	18 (51.4)	21 (60.0)	0.840
100-150 months	12 (35.3)	11 (31.4)	11 (31.4)	
>150 months	4 (11.8)	6 (17.1)	3 (8.6)	
Type of cerebral palsy, n (%)				
Spastic quadriplegia	32 (80.8)	28 (77.8)	30 (80.5)	0.714
Spastic diplegia	4 (19.2)	8 (22.2)	6 (16.6)	
Birth weight (kg)				
Mean (SD)	2.3 (0.97)	2.1 (0.86)	2.0 (0.99)	0.296
Median (range)	2.5 (0.9-4)	1.8 (0.9-3.9)	1.7 (0.9-4.3)	
Weight (kg)				
Mean (SD)	17.3 (7.2)	19.6 (8.5)	17.5 (8.5)	0.445
Median (range)	15.5 (8-35)	18.9 (10-41)	14.5 (9.5-51)	

MNC; Mononuclear cell and MSC; Mesenchymal stromal cell.

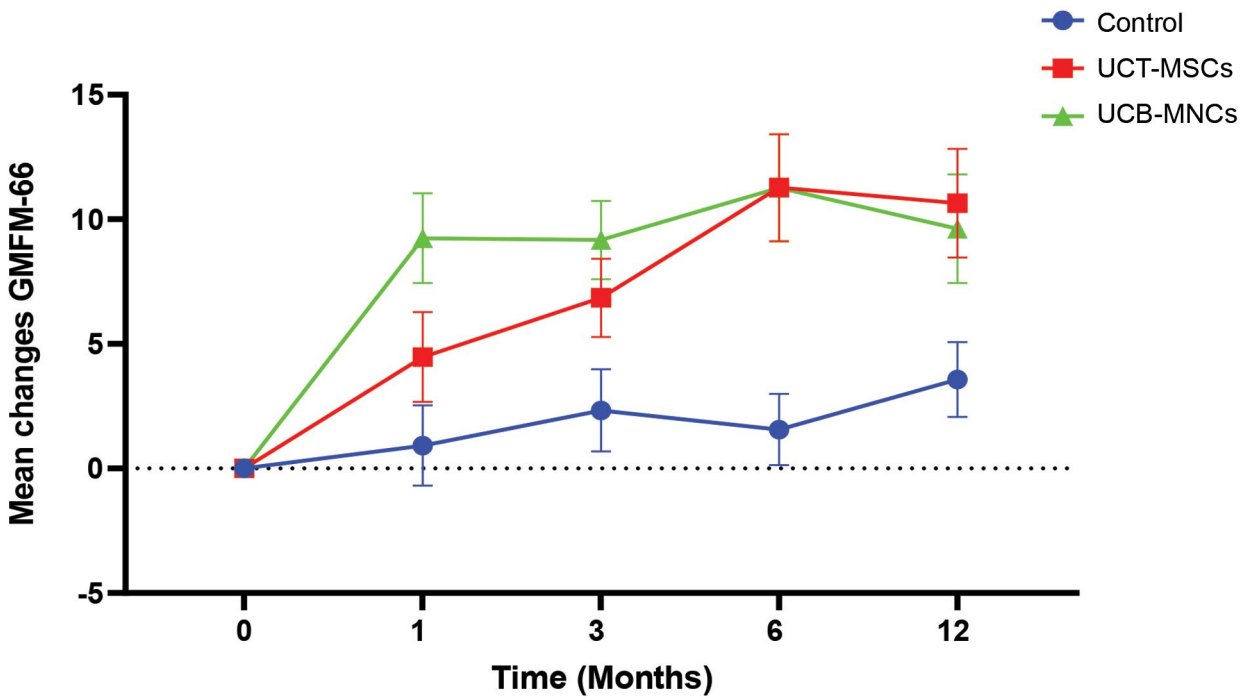

Fig.2: The mean change in GMFM-66, from baseline, at 1ST, 3rd, 6th to 12 months after cell injection in different groups. Green; UC-MNC, Red; UC-MSC, and Blue control.

As discussed in 2021, a systematic review showed that the pattern of biodistribution of MSCs depends on their route of administration (19). Although the current evidence of preclinical stem cell therapy for central nervous system (CNS) diseases indicated that repetitive injections through the IT route have been more beneficial in comparison with other routes, it needs more evaluation and data validation in controlled and blinded clinical studies (20, 21).

Furthermore, co-transplantation of mononuclear cells (MNCs) with UC-MSCs has been suggested for different diseases, such as severe aplastic anemia (22) and myocardial infarction (23). These findings may be useful for the development of more innovative preclinical investigations in CP models.

It is noteworthy that CP is characterized by body movement and muscle coordination impairments while the GMFM-66 has been the most extensively studied measure, consistently providing the best results with the strongest evidence for validity and responsiveness (11). This tool has been validated in numerous studies and systematic reviews, emphasizing its clinical relevance and sensitivity in assessing the efficacy of interventions for CP (2, 24, 25). This makes it the most appropriate tool for evaluating the efficacy of CP interventions.

Our study faces limitations in providing a comprehensive statement that establishes a valid and reliable consensus on the efficacy of utilizing different cell types in the treatment of CP. These limitations include a small sample size, retrospective analysis of the trials, and variations in the cell dosage in the protocols.

Consequently, making a generalized claim about efficacy is challenging. Despite these limitations, our findings suggest potential benefits in comparing the trends, but they must be interpreted with caution. Future studies with larger sample sizes, standardized protocols, and prospective designs are needed to validate these preliminary findings. Additionally, pooled analyses of different studies can provide a more robust assessment and expedite the development of standardized cell therapy protocols.

Our comparative analysis of the efficacy of administrating two different cell types revealed that the MNC injection led to an earlier response in improving the gross motor function of children with CP, whereas MSC application resulted in more sustainable changes. The insight from this study can guide researchers and clinicians in designing cell therapy protocols for future investigations.

Additionally, the identification of other critical variants yet to be defined in cell-based therapy for CP patients, including cell source, cell dose threshold, timing of cell injection, frequency, route of administration, and patient age, underscores the

need for further exploration and optimization of these parameters. These parameters should be considered and optimized for future clinical protocols and it needs a global convergence in addressing the mentioned questions in collaborative research programs.

Our research underscores the importance of continued investigation into refining cell-based therapy protocols for CP treatment. Future studies should focus on elucidating the optimal parameters for cell therapy administration and exploring innovative approaches to maximize therapeutic efficacy. Moreover, exploring the potential benefits of co-transplantation strategies and investigating the biodistribution patterns of administered cells could provide valuable insights for advancing the field of cell-based therapy for CP.

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Authors' Contributions

M.N.; Contributed to project administration, Data curation, and Writing the original draft. M.Z.; Project administration and Writing the original draft. A.M.; Contributed to methodology and Investigation. E.K.; Manuscript writing, Review, and Editing. S.M., M.A.; Contributed to the investigation, Formal analysis, Data curation, and Visualization. M.R.A., M.V.; Conceptualization, Supervision, Project Administration, Writing the original draft, Review and Editing. All authors read and approved the final manuscript.

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