A Preliminary Clinical Study on the Treatment of Cerebral Palsy (CP) Using Umbilical Cord Blood Stem Cells

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[Abstract]

Objective: To evaluate the effects of treating cerebral palsy with umbilical cord blood stem cell therapy.

Methods: Twenty-six patients received cord blood stem cell transplantation through intrathecal and intravenous injections. Signs and symptoms were recorded and the Gross Motor Function Measure was used to evaluate the change in motor function of patients after one round of treatment. The Ashworth Classified Measure was used to evaluate differences in muscle tone before and after treatment. Lab examinations of T-cell subtypes were used to evaluate the immune function of patients.

Results: The motor function of patients improved with statistical significance ($P<0.01$); however, T cells subtypes revealed no significant change ($P>0.05$) after stem cell treatment.

Conclusion: Using cord blood stem cells to treat CP can improve gross motor function while not causing any harmful effects to immune-system functions.

[Key words]

Umbilical cord blood stem cells; cerebral palsy; T-lymphocyte subsets

Introduction

Cerebral palsy (CP) refers to non-progressive brain lesions developing before, during, and after birth. It is characterized by motor dysfunction and posture abnormalities. Human umbilical cord blood contains not only a large number of hematopoietic stem cells and progenitor cells (HSC/HPC), but is also rich in mesenchymal stem cells, which have the ability of multi-directional differentiation. The proportion of viable stem cells in cord blood is much higher than in adult's bone marrow and peripheral blood. There is evidence that cord blood stem cells are potentially capable of differentiating into neural cells [2]. Our observations of the application of cord blood stem cells in treating patients with CP imply that cord blood stem cells can improve the gross function of children with cerebral palsy. In this study, the daily-life capabilities of patients also improved with no harmful side effects to the immune system.

Basic information and Methods

1． Basic information

Twenty-six cases of children with cerebral palsy were involved in this study. Their birth weights ranged from 0.53 to 6.12 kg with the average being 2.60kg; gestational weeks at birth ranged from 25 to 42 weeks with the average being 35.85 weeks. Of the 26 cases, 15 were spastic CP, one athetosis CP, five mixed types, and five unclassified types. No cases of tonic CP, ataxia CP, or trembling/hypotania CP were involved. Traceable causes included premature birth, low birth weight, hypoxia, nuclear jaundice, and congenital brain malformation. Among the 26 cases, there were six cases of premature birth plus low birth weight (gestational weeks<37 weeks), two cases of solely low birth weight (birth weight<2.50 kg), one case of mere premature birth, five cases of suffocation, two cases of kernicterus, four cases of congenital brain malformation (hydrocephalus, head deformity), two cases of postnatal purulent

meningitis, and two cases of unknown etiologies. In the 26 cases studied, patients displayed a range of symptoms including mental retardation, language dysfunction, and epilepsy. All patients underwent EEG, brain CT or MRI, and ECT. Abnormal EEG and abnormal brain CT or MRI results were found in 7 of the 20 cases. Positive findings using these images included softened white matter, brain dysplasia, macrogyria, microcephalia, hydrocephalus, myelin malformation, callosal agenesis, and ventriculomegaly (see figure 1-4). Two cases had a normal EEG and brain MRI, and no mental retardation or language dysfunction was found.

Table 1: General background information on the 26 patients involved in this Study

Number of Patients	Average Birth Weight (kg)	Average Gestational Period (week)	Types of CP			
			Spastic	Athetosis	Mixed	Unclassified
26	260	3585	15	1	5	5

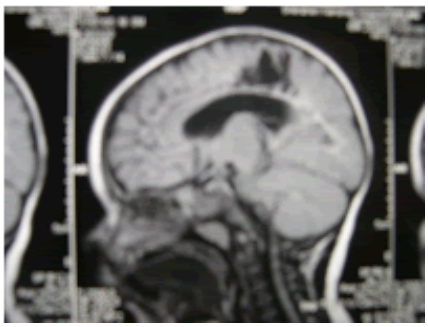


Figure 1

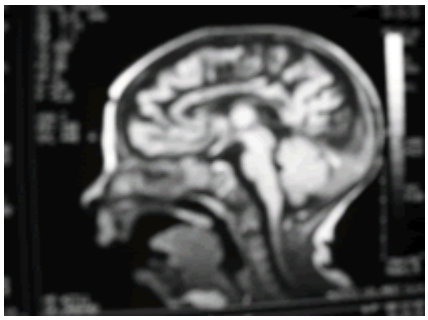


Figure 2

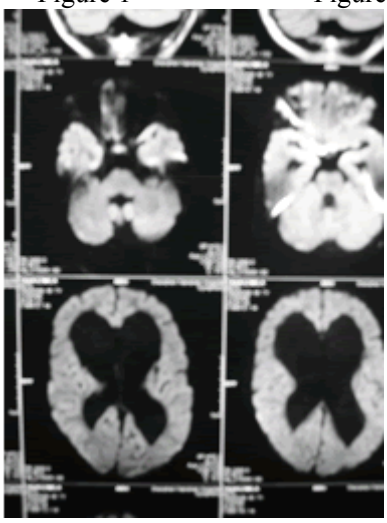


Figure 3

Figure 4

2． Isolation and Culturing of Cord Blood Stem Cells

In order to isolate the cord blood mononuclear cells (MNCs), density gradient centrifugation was utilized.

3. Cord Blood Stem Cells Tests

3.1 Cell purity and cell activity

Mononuclear Cells percentage > 95%; Cell Activity Detection percentage > 95%.

3.2 Identification of cell surface markers

(A) Hematopoietic stem cells: CD34 (+), CD133 (+).

(B) Mesenchymal stem cells: CD44 (+), CD105 (+).

3.3 Detection of exogenous substances

(A) During the cultivation and distribution of stem cells, pre-testing was done to verify the absence of bacteria fungi, mycoplasma, and Chlamydia.

(B) Virus indicators for HBV, HCV, HIV, and RPR were normal.

(C) Endotoxin and pyrogen indicators were normal.

4. Treatment methods

The transplantation for the treatment of cerebral palsy was made via intrathecal infusion in combination with intravenous infusion. Intrathecal infusion via lumbar puncture was the primary method, with one intravenous infusion also being used for each patient's treatment cycle. The interval between each infusion was one week. Total infusions for the one course of treatment were four. During the four weeks, rehabilitation exercises were regularly provided.

5. Evaluation of Treatment Effectiveness

Treatment effectiveness was evaluated using a Gross Motor Function Measure (GMFM) scale developed by Russell, a modified Ashworth grade, as well as the GESELL Evaluation Scale.

6. Statistical Analysis

Paired T-tests were used in statistical analysis of normally distributed data. Ranked data were analyzed using rank tests. The SPSS 11.5 statistical software package was used for statistical analysis in this study.

Results:

1. In comparing T cell subsets of the patients with cerebral palsy (n = 20) before and after transplantation, we can see from the clinical data that the total number of T lymphocytes in peripheral blood and the proportion of each T cell subgroup were almost the same as normal values. In addition, the total number of T lymphocytes in peripheral blood and the proportion of each T cell subgroup did not change significantly after the first course of treatment. In the assessment of the difference of T lymphocytes in peripheral blood and the proportions of each T cell subgroup by statistical analysis, no significant statistical differences were found ($P > 0.05$),

suggesting that cord blood stem cell transplantation in children with cerebral palsy does not have an adverse impact on the immune function.

Table 2: The Total Number of T-Lymphocyte Before and After Treatment in 20 CP Patients

Before Treatment	After Treatment		Total
	Normal	Abnormal	
Normal	12	6	18
Abnormal	2	0	2
Total	14	6	20

2. Changes in Motor Function of Children with Cerebral Palsy before and after Transplantation:

An assessment of motor function in children with CP before and after each treatment was done using the Gross Motor Function Measure (GMFM) scale developed by Russell.

There are a total of 80 motor function indicators in GMFM that can be divided into five functional aspects: 1) supine and prone position, turning activities, the remaining of the original reflex, and establishment of static reflex; 2) kneeling and crawling; 3) sitting and balance reflex; 4) standing; 5) walking, running, and jumping activities.
A mark was given to each indicator according to the degree to which the action was completed. Full inability was given 0 points; <10% completion = 1 point; completed 10% ~ 90% completion = 2 points; >90% completion = 3 points.

Final scores were calculated in three ways:
1) Final score for each functional aspect (score for each functional aspect × 100).
2) Total score: a total of all final scores for each functional aspect added together.
3) Actual score: total score divided by the number of functional aspects evaluated.

Table 3: A Comparison Assessing Motor Function by GMFMs in Children with Cerebral Palsy before and after Treatment ($\bar{x}\pm s, n=20$)

Group/Aspect	Before Treatment	After Treatment
A	25.47±4.28	29.32±4.31*
B	17.29±5.09	18.74±5.05*
C	10.26±4.04	10.84±4.11
D	7.42±3.68	7.89±3.75
E	8.21±4.48	11.68±5.86*
Total scores	13.83±2.03	15.69±2.19*

Note: comparing with before the treatment, *$P<0.05$。

From Table 3, after one course of treatment, it can be observed that motor function improved when compared with function scores before treatment. Especially for A, B, and E functional aspects, the differences were statistically significant (P <0.05).

3. Calf Muscle Tension Changes in Children with Cerebral Palsy before and after Transplantation

Assessment of calf muscle tension was made by using the modified Ashworth grade. Table 4 shows that the difference of calf muscle tension before and after treatment is not statistically significant (P>0.05), even though some patients displayed improvement to various extents. Some factors that could help explain these findings are a small sample size and the fact that stem cell therapy may not be able to improve muscle tension in a short period of time.

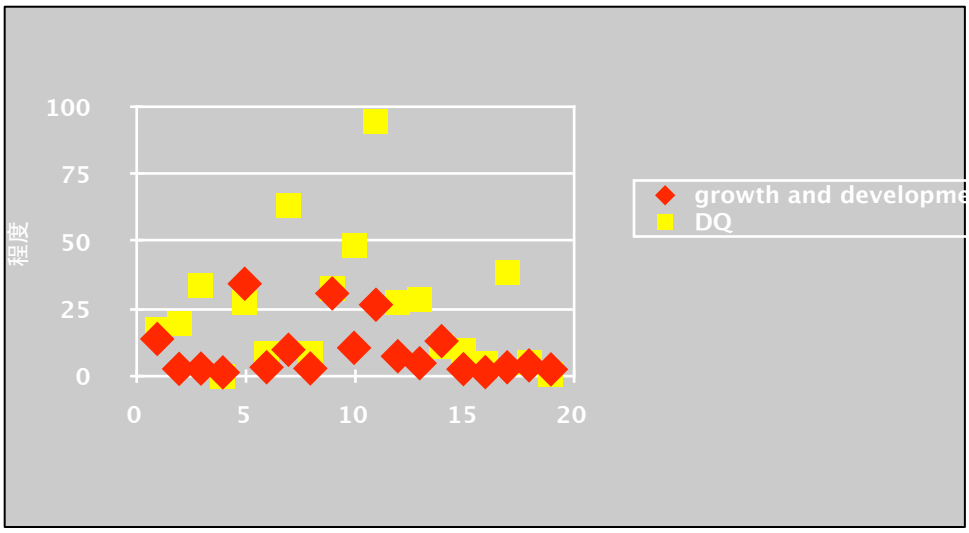
Table 4: Calf Muscle Tension Changes in Children with Cerebral Palsy before and after Transplantation

Muscle tension	Before the treatment	After the treatment
0	2	5
1	2	0
1+	4	5
2	5	6
3	6	4
4	1	0
Total	20	20

4. Statistical Results in Assessment of Intelligence Capacity of Children with CP

All 20 patients treated with the stem cell therapy in this assessment show that the patients' growth and development level was 1.9 months - 34.8 months, the average being 9.86 months. Development quotient (DQ) was 0.4-95 points with an average of 25.72 points. Of the 20 cases, there were two cases of borderline type, one case of autistic type, and the others were medium-to-severe CP cases. Post-treatment intelligence capability assessment is still in process.

Figure 4: Results in Assessment of Intelligence Capacity of Children with CP



Discussion:

1) Neural stem cell transplantation does not have an adverse effect on the immune function of children with cerebral palsy.

2) Neural stem cells can effectively improve gross motor functions of children with cerebral palsy.

3) The assessment results of muscle tension and intelligence capability imply that there were no significant effects shortly after treatment, and therefore further studies in these areas are needed.

Case Study Report

Timea, a Hungarian girl of four and a half months old, has severe hydrocephalus. After a ventriculoperitoneal shunt, there was still accumulated water/hydrocele in 40% of the brain. Her intelligence, movement, growth, and development levels were comparable to that of a 3.5-week-old child. Her left hand was closed at all times, her eyes were not able to follow passing objects, and she was not able to stand on her four limbs. After six injections of stem cells and regular rehabilitation training once daily, she was able to move her limbs and eyes with greater accuracy, she started eating and sleeping well, and she began smiling. After stem cell therapy, she was appraised as 12-week-old in terms of intelligence, movement, growth, and development. Her doctors were surprised at the notable results when she arrived back in her hometown. Her progress attracted the media's attention shortly thereafter.

The girl's mother, an associate professor of economics at the University of Budapest, brought her daughter back for a second treatment along with five other children with CP. At the time of publication, the 10-month-old girl exhibits the intelligence, movement, growth, and development levels of a 6-month-old child.

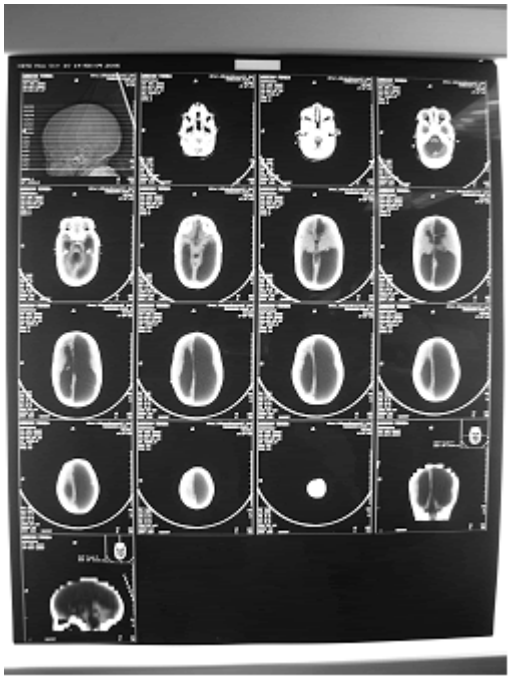


Figure 7 CT photo 2 months after birth

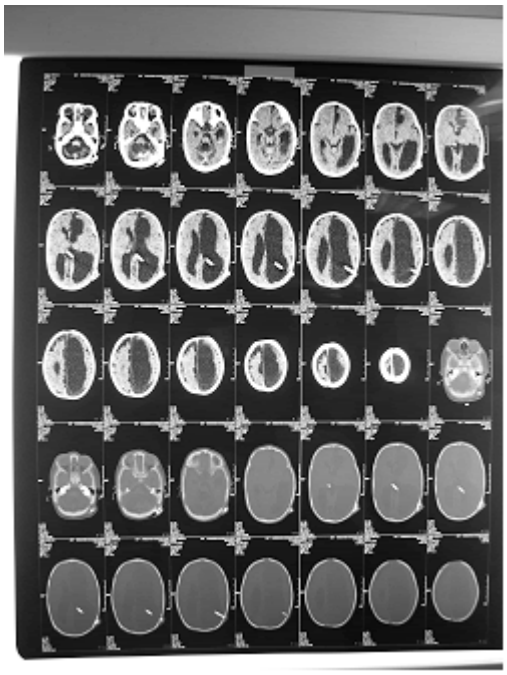


figure 8: ventriculoperitoneal shunt 4.5 months after birth

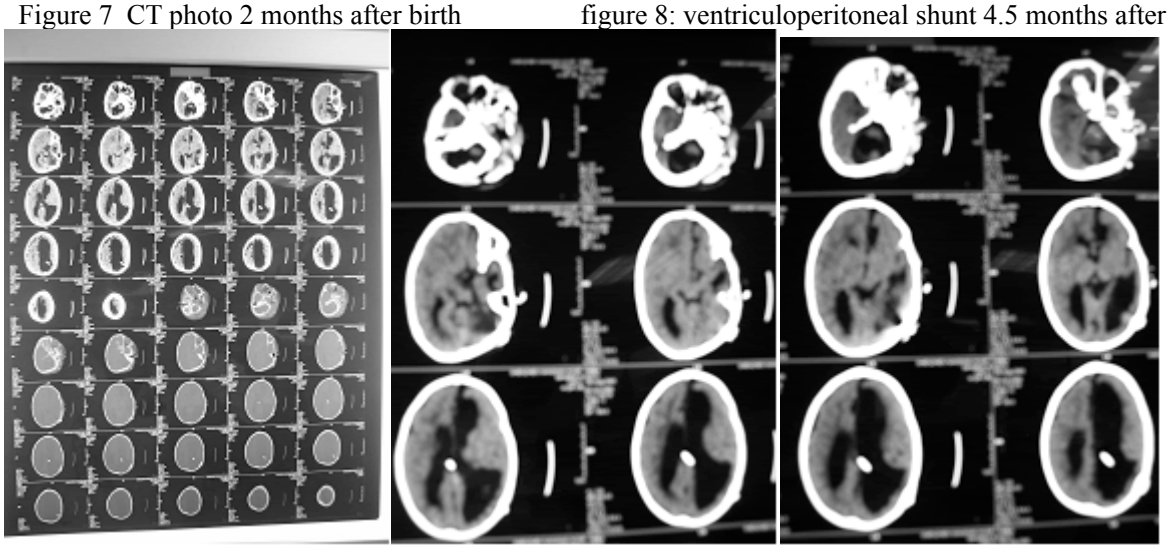


Figure 9 CT scanning for the third time