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Human allogeneic AB0/Rh-identical umbilical cord blood cells in the treatment of juvenile patients with cerebral palsy

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Abstract

Background aims. The term "cerebral palsy" (CP) encompasses many syndromes that emerge from brain damage at early stages of ontogenesis and manifest as the inability to retain a normal body position or perform controlled movements. Existing methods of CP treatment, including various rehabilitation strategies and surgical and pharmacological interventions, are mostly palliative, and there is no specific therapy focused on restoring injured brain function. *Methods.* During a post-registration clinical investigation, the safety and efficacy of intravenous infusion of allogeneic human leukocyte antigen (HLA)-unmatched umbilical cord blood (UCB) cells were studied in 80 pediatric patients with cerebral palsy and associated neurological complications. Patients received up to 6 intravenous infusions of AB0/Rh-identical, red blood cell–depleted UCB cells at an average dose of 250×10^6 viable cells per infusion. *Results.* Patients were followed for 3–36 months, and multiple cell infusions did not cause any adverse effects. In contrast, in most patients who received four or more UCB cell infusions, positive dynamics related to significant improvements in neurological status and/or cognitive functions were observed. *Conclusions.* The results confirm that multiple intravenous infusions of allogeneic AB0/Rh-identical UCB cells may be a safe and effective procedure and could be included in treatment and rehabilitation programs for juvenile patients with cerebral palsy.

Key Words: *brain injury, cell therapy, cerebral palsy, umbilical cord blood*

Introduction

The term "cerebral palsy" (CP) encompasses various syndromes that emerge from brain damage at early stages of ontogenesis and manifest as the inability to retain a normal body position or perform controlled movements. Motor disturbances (pareses, coordination disorders, forced movements, etc.) may be associated with changes in mental status, speech, vision, hearing, and sensitivity disorders. In childhood development, the clinical manifestations of CP can vary. Furthermore, if CP co-exists with additional pathological conditions such as hydrocephalus, infectious diseases, intoxications or traumatic brain injury, the disease progresses.

According to estimates made by many authors in various countries, the incidence of CP ranges from 1 to 8 per 1000 newborns and is increasing. However, existing methods of CP treatment, including various rehabilitation strategies and surgical and pharmacological interventions, are mostly palliative, and there is no specific therapy focused on restoring injured brain function.

Human umbilical cord blood (UCB) is enriched in various stem/progenitor cell populations. These cells are capable of forming a complete hematopoietic system if transplanted into experimental animals and of restoring hematopoiesis in leukemia patients [1,2]. UCB also contains embryonic-like stem cells, mesenchymal stem cells, endothelial progenitor cells and unrestricted somatic stem cells that can form various types of tissues including those of mesodermal, endodermal or ectodermal lineages [3–8]. Furthermore, UCB cells produce numerous cytokines

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and immunomodulatory and neurotrophic factors that can modulate brain "plasticity" and contribute to functional brain repair [9–12].

Several pre-clinical animal studies as well as recent clinical investigations suggest that UCB cells are amenable to the treatment of a wide spectrum of diseases [13–15]. In particular, regenerative cell therapy holds promise in various pathological neurologic conditions including CP and traumatic and ischemic brain injuries [16–21]. The use of UCB cells might accelerate reparative processes by replacing, regenerating or intensifying the biological activity of damaged cells to restore or improve brain function.

In this study, we verified the safety and therapeutic efficiency of intravenous infusion of allogeneic human leukocyte antigen (HLA)-unmatched AB0/Rh-identical UCB cells in pediatric patients suffering from diverse forms of CP and concomitant injuries to the nervous system.

Methods

Umbilical cord blood collection, processing and cryopreservation

Cord blood was obtained after obtaining informed consent during full-term normal deliveries from healthy women at the Federal Center of Obstetrics, Gynecology and Perinatology, Moscow, Russian Federation. Blood samples (40–220 mL) were aseptically collected in blood-collection bags containing 35 mL of citrate phosphate dextrose (CPDA-1) anticoagulant and processed within 24 hours. Red blood cell-depleted/plasma-reduced nucleated cells were isolated by sedimentation [22], re-suspended in autologous plasma with 10% dimethylsulfoxide and 1% dextran-40, aliquoted in 4-mL cryovials and cooled to −90°C using a controlled-rate freezer. Therapeutic doses were engineered to contain 3–4 × 10^8 cells per vial (Table I). During quarantinization in the vapor phase of liquid nitrogen, all of the samples were tested for HIV-1/2, hepatitis B and C, HTLV-1/2, HSV-1 and -2, CMV and syphilis and characterized by AB0/Rh, CD34/CD45-positive cell content [23,24] and sterility.

Therapeutic cell preparation

After thawing in the water bath at +37°C, UCB cells were washed free of cryoprotectant in an excess of physiological saline containing 2.5% human serum albumin and 5% dextran-40 and re-suspended in 50 mL of the same solution at a density of 250 ± 15 × 10^6 viable cells. Cell viability was estimated by routine trypan blue testing and/or by flow cytometry with 7-aminoactinomycin D vital dye [24]. The cell suspensions were then transferred into plastic infusion bags and used within 1 hour.

Patients

Data were collected from patients treated from January 2011 to December 2013 as participants in a post-registration study of medical technology, "The use of umbilical cord blood nucleated cells in the treatment and rehabilitation of patients with neurodegenerative diseases, traumatic and perinatal brain injuries" that was approved for clinical application by the Russian Federal Service on Surveillance in Healthcare and Social Development (Roszdravnadzor, Permission #2009/387). The treatment was provided as a part of licensed medical practice at the Federal Center of Obstetrics, Gynecology and Perinatology of the Russian Ministry of Health.

The cell therapy group comprised 80 pediatric patients (1–12 years old, 53 male, 27 female) with a clinically confirmed diagnosis of CP, including 40 with spastic quadriplegia, 24 with spastic di- or hemiplegia and 16 with other forms of CP. The patients' characteristics are summarized in Table II. In most patients, CP was associated with other pathological conditions, such as epilepsy ($n = 20$), congenital hydrocephalus ($n = 7$), partial atrophy of the optic nerve ($n = 10$) and other injuries ($n = 5$). Most children ($n = 55$) had a delay in physical and mental development.

In accordance with the exclusion criteria, all of the patients were free of compromised vital functions, a prior history of severe allergic or transfusion reactions, active focal or systemic infections, acute somatic conditions, coagulopathy or significant alterations in blood analyses and urine tests, and severe psychiatric disorders.

Table I. Parameters of cord blood units and isolated UCB cells.

Parameter	Mean	SD	Median	Sum	Min	Max
Blood volume, mL	91.8	27.8	89.0	—	40.0	190.0
TNC initial, × 10^6	1484	590	1365	—	374	3957
TNC after separation, × 10^6	1312	503	1222	—	363	3203
$CD34^+$ cells, %	0.29	0.16	0.25	—	0.04	1.38
Number of TDs per UCB unit	3.8	1.5	3.0	803	1	9
Nucleated cells per TD, × 10^6	320	23	319	—	269	397

The number of analyzed UCB samples, $n = 212$.Max, maximum value; Min, minimum value; TD, therapeutic dose; TNC, total nucleated cell count.

Table II. Patient characteristics.

Characteristic	*n*	%
Age at first infusion (years)		
1–2	16	20.00
2–4	19	23.75
4–6	17	21.25
6–8	21	26.25
8–12	7	8.75
Sex		
Male	53	66.25
Female	27	33.75
Diagnosis (ICD-10)		
G80, CP	80	100.00
G80.0, spastic quadriplegic CP	40	50.00
G80.1, spastic diplegic CP	20	25.00
G80.2, spastic hemiplegic CP	4	5.00
G80.4, ataxic CP	4	5.00
G80.3, dyskinetic CP	1	1.25
G80.8/G80.9, other CP/CP, unspecified	11	13.75
Complications		
Congenital hydrocephalus	7	8.75
Symptomatic epilepsy	20	25.00
Optic nerve atrophy	10	12.50
Developmental delay	55	68.75
Other brain injuries	5	6.25

ICD-10, *International Classification of Diseases*, 10th revision.

Cell infusion

After pre-medication with Clemastine (Tavegyl, Novartis Consumer Health; 0.025 mg/kg body weight, intravenously), the cells were introduced into patients via a peripheral vein using a transfusion system with nylon filter ("dropper"); the duration of the procedure typically did not exceed 30 min. Vital signs (pulse, respiratory rate, skin color, etc.) were monitored during the infusion and for 2–3 h after the procedure.

Treatment plan

Informed consent forms signed by the parents were obtained for each patient before initiating treatment and for each UCB cell infusion. During the initial treatment, all of the patients received two infusions of UCB cells with 2- to 3-week intervals (preferably, from a single UCB sample). In 55 children, the courses of cell therapy were repeated 3–6 and 6–12 months later (in most cases, cells were selected from among one or two appropriate blood units reserved in a computer database at enrollment).

Evaluation of therapeutic benefit

After enrolling and before each UCB cell infusion, the patients' neurological and mental (if applicable) statuses were evaluated by a medical commission including a pediatrician, pediatric neurologist and medical psychologist. Measurement of muscular tone according to the modified Ashworth scale [25] and strength [26] were applied to estimate the severity of the neurological deficits. Additional tests including hand dynamometry and the number of steps and squats performed by the child independently during 20 seconds were used to define the level of physical development. Abilities and limitations in motor functions were characterized according to the Gross Motor Function Classification System (GMFCS) scale [27].

Statistical analyses

Descriptive statistics were used to characterize cord blood units (blood volume, total nucleated cell content before and after processing, $CD34^+$ cell content, etc.). Because of the limited number of patients and significant variability in patients' ages and severity of neurological and/or physical impairments, the effects of cell therapy were calculated as the relative change in the corresponding parameter compared with its initial value. *t* tests for paired samples and Wilcoxon matched pair tests (STATISTICA 6.0 for Windows, StatSoft Inc.) were used to measure the significance of the differences between the pre- and post-treatment values. $P \leq 0.05$ was considered significant.

Results

More than 250 UCB units were collected and processed, of which 212 were approved for clinical application. Samples containing fewer than 5×10^8 nucleated cells and/or displaying positive infectious agent/bacterial culture test results were discarded. Finally, 803 appropriate therapeutic doses containing a goal dose of $3–3.5 \times 10^8$ nucleated cells (mean, $3.2 \pm 0.23 \times 10^8$; median, 3.18×10^8) were prepared, transferred into liquid nitrogen and stored until use. The majority of CBUs were processed and cryopreserved before the initiation of this study as a separate collection. In case of need, it was replenished to the level of approximately 300–350 therapeutic doses. According to our experience, this was enough for subsequent selection of appropriate samples for patients with even rare AB0/Rh combinations.

The blood units' and cell characteristics are summarized in Table I. The viability of post-processed UCB cells was high (99.2 ± 0.6%). The post-thaw cell viability, as determined by 7-aminoactinomycin D staining, remained high for lymphocytes (98.2 ± 1.3%), monocytes (98.5 ± 1.2%) and hematopoietic (CD34/CD45-positive) cells (97.1 ± 1.8%). The moderate TNC loss

observed was mostly related to the decreased viability of granulocytes (80.1 ± 5.8%).

During the last 3 years, 80 pediatric patients (1−12 years old; 53 male, 27 female) with a clinically confirmed diagnosis of CP (G80 according to *International Classification of Diseases*, 10th revision) received 272 intravenous infusions of allogeneic RBC-depleted plasma-reduced AB0/Rh-identical UCB cells at an average dose of 250 × 10^6 cells per infusion.

In terms of diagnosis, severe forms of CP were prevalent (40 patients were tetraplegic). According to a detailed analysis of the medical documentation, the most frequent possible causes of brain injury were fetal hypoxia/ischemia (42%), preterm delivery (18%), and birth trauma (14%). In all of the cases, the previously applied methods of treatment and/or rehabilitation were ineffective. Additionally, none of the patients included in this study had autologous UCB cells banked at CryoCenter or at another Russian cord blood bank.

In accordance with individual treatment plans and the stage of treatment (initial or extended), patients received one ($n = 7$), two ($n = 18$), three ($n = 19$), four ($n = 15$) or five ($n = 14$) cell infusions. Seven patients had acquired six UCB cell introductions (two initial cell infusions followed by four cell therapy courses with a 4- to 6-month interval). Children who interrupted the treatment after the first cell infusion (according to parents' decision), did not arrive for scheduled examinations or were not observed for more than 3 months after initial treatment were excluded from statistical analysis. In the remaining 55 patients, the follow-up period ranged from 3 months to 3 years.

Cell administration was well tolerated, and no acute or delayed adverse reactions (allergic reactions, headache, waist pain, fever, vomiting, etc.) were registered. In our results, positive dynamics were observed in 38 patients (69.1%) receiving cell therapy. The improvement in neurological status was characterized by a decrease in the pathological muscular tone in one or more affected limbs, an increase in muscular strength, and a reduction in epileptic paroxysms. Amelioration in the mental sphere (speech, memory, attention, intellectual and emotional development) was noted in 29 (52.7%) cases. Twenty-three children (41.8%) demonstrated progress in both the physical and mental arenas. Seventeen patients (30.9%) did not change their neurological and/or mental state during post-treatment observation. Negative dynamics were not revealed in any of the children receiving cell therapy.

The results of cell therapy on muscular tone, strength and physical development indices are presented in Figures 1−3. A subgroup of patients enrolled after January 2012 ($n = 38$) who received two or more UCB cell infusions was evaluated according to GMFCS scale (Table III). To date, 18 (47.4%) of the patients have displayed significant progress in general motor activities by acquiring the ability to sit without support (Level IV) and to walk with (Level III) or without (Levels II) assistance.

Detailed statistical analysis showed that the results of the treatment depended on at least three factors: patient age, the severity of the brain damage and the number of UCB cell infusions. For instance, in patients aged 1−2 years ($n = 9$) with the most severe form of CP (six with spastic quadriplegia), cell

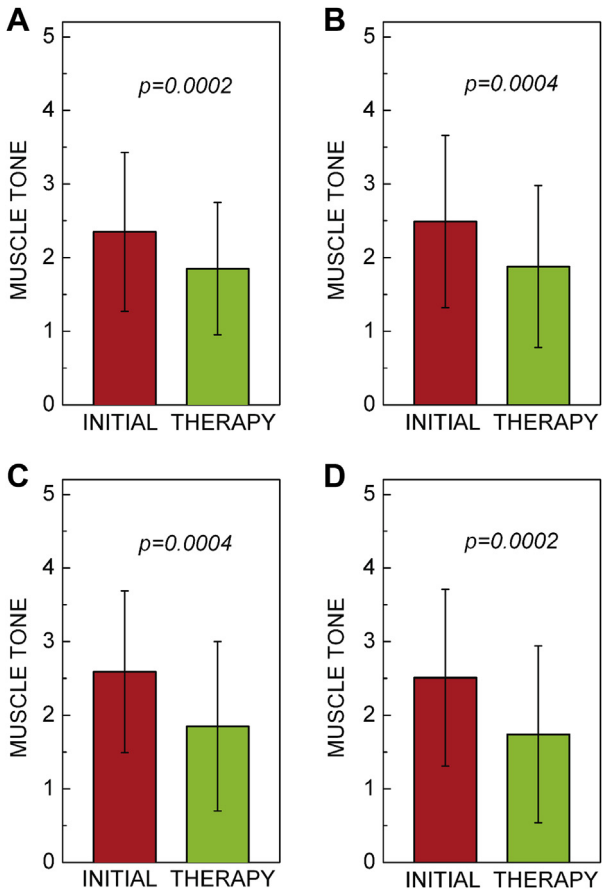

Figure 1. Effect of UCB cell therapy on muscle tone in patients with cerebral palsy (estimation according to modified Ashworth scale). A, Right hand; B, left hand; C, right leg; D, left leg. Data are presented as the mean ± SD (m ± SD; $n = 36$). Spasticity is an important cause of neurological disability. Muscle tone is defined by the resistance of a muscle being stretched without resistance. The modified Ashworth scale has 5 points: 0 = no increase in tone; 1 = slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected part(s) is moved in flexion or extension; 2 = slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the reminder (less than half) of the range of movement (ROM); 3 = more marked increase in muscle tone through most of the ROM but affected part(s) easily moved; 4 = considerable increase in muscle tone, passive movement difficult; 5 = affected part(s) rigid in flexion or extension.

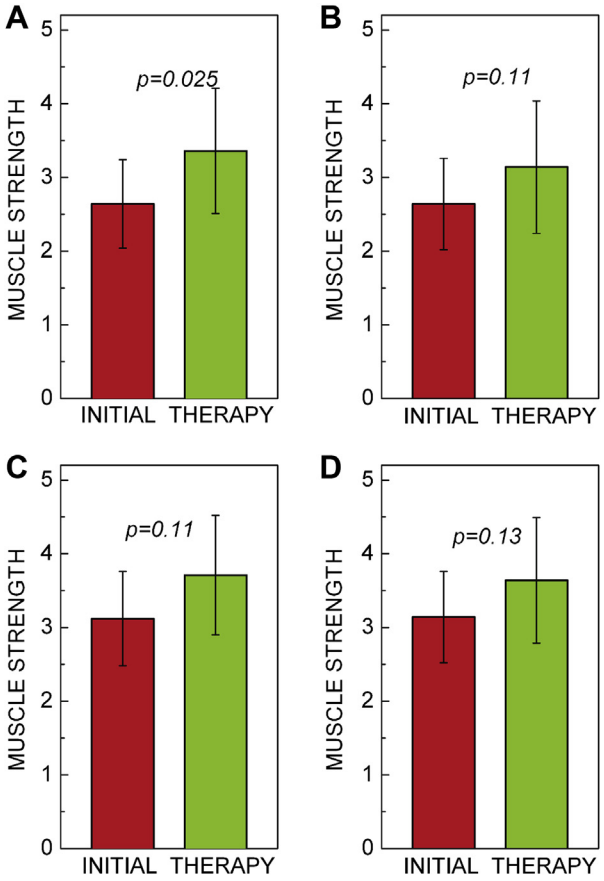

Figure 2. Effects of UCB cell therapy on muscle strength (estimation according to McPeak scale). A, Right hand; B, left hand; C, right leg; D, left leg. Data are presented as mean ± SD. Of 55 patients, 23 failed to pass muscular strength test due to severe neurological impairment. Significant improvement of this parameter (by one or more points in one or more limbs) was observed in nine children. The patient's effort is graded on a scale of 0–5: grade 5: muscle contracts normally against full resistance; grade 4: muscle strength is reduced, but muscle contraction can still move joint against resistance; grade 3, muscle strength is further reduced such that the joint can be moved only against gravity with the examiner's resistance completely removed; grade 2, muscle can move only if the resistance of gravity is removed; grade 1: only a trace or flicker of movement is seen or felt in the muscle or fasciculations are observed in the muscle; grade 0: no movement is observed.

therapy led to improvement in the emotional/mnestic sphere (babble, initial formation of phrasal speech, recognition, etc.), a significant decrease in pathological muscular tone and an extension of locomotor activity in approximately half of cases (55.6% and 55.6%, respectively).

In the group of patients aged 2–6 years old (n = 21; 13 with spastic quadriplegia, 4 with spastic diplegia or hemiplegia), positive dynamics were observed more often. Therefore, a decrease in pathological muscle tone and augmentation of physical development indices (muscular strength, the number of steps and squats) were achieved in 13 (61.9%) patients. Similar results (n = 14; 66.7%) were obtained after analyzing changes in the mental sphere. Ten children (47.6%) demonstrated improvement in both arenas. Additionally, in two patients in whom their CP was complicated by epilepsy, the frequency and severity of paroxysms decreased without changes in their anticonvulsant dosages. In one child, the epileptic seizures were completely abolished.

In children older than 6 years old (n = 20), positive dynamics were observed predominantly in neurological status (normalization of pathological muscle tone, increase of muscular strength) and physical (arm force and the number of steps and squats) development (70.0%) with a slightly smaller effect on mental (attention, memory and intellectual) development (50.0%). In three of three patients with epilepsy, the manifestations of the disease were eliminated.

These findings indicate that repeated intravenous infusions of allogeneic HLA-unmatched AB0/Rh-identical UCB cells to pediatric patients with CP is safe and is an effective intervention in most cases. At least partial improvement in neurological status, physical activity and/or intellectual development was achieved in approximately 70% of cell therapy patients.

Discussion

It may seem that UCB cell therapy is most effective in CP patients aged 2–6 years. However, analysis of the correlations between the effectiveness of treatment and other factors such as the severity of the disease and the number of cell infusions indicates that patient age is not the main predictor of cell therapy success. The group of "non-responders" consisted predominantly of patients suffering from the most severe forms of CP complicated by other brain damage and patients receiving no more than two UCB cells infusions (i.e., completed only the initial stage of cell therapy).

After two UCB cell infusions, neurological improvement was recorded in 8 of 19 patients (42.1%). In children who received three cell infusions (n = 15), positive dynamics were achieved in 40% (physical sphere) and 47% (mental sphere). The best results were obtained in response to five and more treatments; improvements in neurological/physical and mental statuses were noted in 85.7% and 100% of patients, respectively (Figure 4). The effectiveness of the therapy was positively correlated with the number of UCB cell infusions and was statistically significant (coefficient of correlation, r = 0.48; P = 0.0024). A similar correlation (r = 0.48, P = 0.0054) was established after analyzing changes

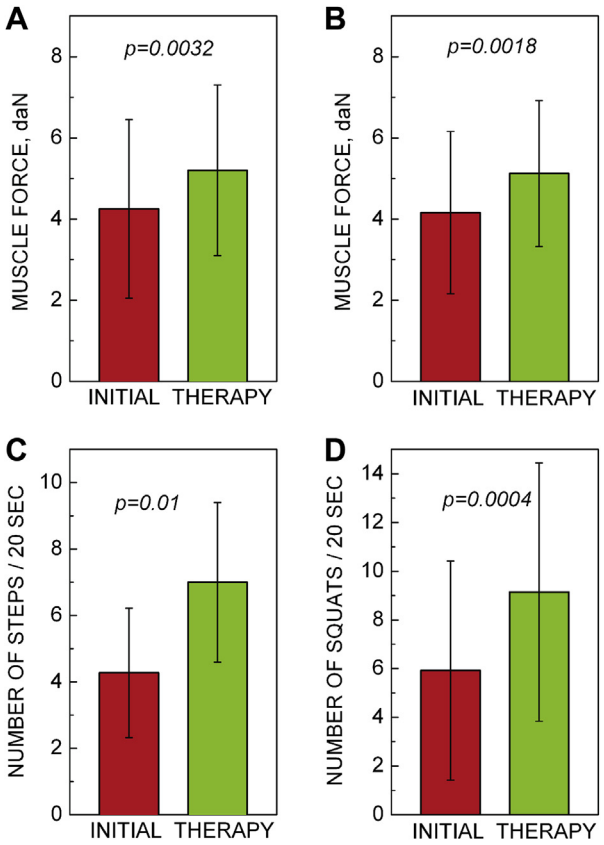

Figure 3. Effects of UCB cell therapy on physical development indices. A and B, results of hand dynamometry (A, right hand; B, left hand); C and D, number of steps and squats performed by patients independently during 20 seconds, respectively. Data are presented as mean ± SD for 15 patients (aged 4.6–7.5 years) with the ability to hold and squeeze the dynamometer and/or walk on one's own before initiating treatment.

in physical development as assessed using the GMFCS scale. Therefore, an overwhelming majority of "responders" had been administered at least four cell infusions (Table III). In the group of patients who did not respond to cell therapy with significant motor function improvement ($n = 19$), 12 were tetraplegic, and in eight cases, CP was accompanied by epilepsy. The last observation indicates that combined severe brain injuries are less sensitive to this type of treatment.

Since 1968, UCB has been successfully used mostly to reconstitute the hematopoietic system in patients with hematological malignancies, and more than 25,000 UCB transplants have been performed worldwide [1,2]. Recently, several preclinical and clinical studies have been conducted supporting the possibility of using UCB cells in the treatment of patients with non-malignant diseases such as type I diabetes mellitus, stroke and myocardial infarction. However, in the case of blood disorders, HLA-matching and recipient preconditioning are obligatory to achieve clinical effect, and the issue of permanent graft survival remains unclear in non-malignant diseases (including those of the central nervous system).

In the 1930s and 1940s, UCB was successfully used for transfusion instead of donor blood [28]. Because HLA-matching was not available at that time and no negative consequences were reported, it was discovered that UCB transfusion does not require conditioning of the recipient. More recently, UCB was used to treat patients with anemia caused by severe malaria [29]. More than 100 children with severe anemia received transfusions of AB0-matched blood (mean dose 85 mL) without HLA typing. The absence of adverse events, including graft-versus-host disease (GVHD), led to the conclusion that UCB can be used for transfusions if donor blood is unavailable. The results of 413 HLA-unmatched UCB unit transfusions without preconditioning to 129 patients suffering from malignant and non-malignant diseases were summarized by Bhattacharya [30]. The absence of GVHD and other immune consequences after UCB administration were confirmed in later publications [31–33].

On the basis of previous studies, it can be concluded that transfusions of UCB to patients with a normal immune system is safe and not associated with serious consequences, and "the worst a cord blood transplant will do is do nothing" [34].

According to the international resource www.clinicaltrials.gov, during the past decade, both the safety and efficacy of autologous and allogeneic UCB cells in the therapy of neurological diseases were investigated in several clinical centers worldwide. From March 2004 to December 2009 under the Pediatric Blood and Marrow Transplantation Program at Duke University, the safety of intravenously infused autologous UCB cells was examined in children with acquired neurological diseases. During the study period, a total of 184 patients (140 with CP) received 198 transfusions of UCB cells at a dose $>10^7$ cells/kg body weight. To reduce the risk of adverse events, UCB cells were washed free of cryoprotectant and infused after premedication with acetaminophen, diphenhydramine and methylprednisolone. Post-transfusion complications only occurred in three patients [35,36].

The results of the intravenous infusion of autologous UCB cells to 20 CP patients aged 2–10 years (11 quadriplegics, six hemiplegics and three diplegics) are presented in the publication by Lee *et al.* [37]. Cryopreserved autologous UCB cells were thawed at the bedside and infused intravenously at a mean dose of 5.5 ± 3.8 (0.6–15.65) × 10^7 cells/kg body weight with subsequent rehydration therapy. After 6 months, neurodevelopmental improvements occurred in 25% of patients, predominantly in

Table III. Effects of UCB cell therapy on patients' gross motor functions.

Patient	No. of cell infusions	Age at first infusion (years + months)	Age at last examination (years + months)	GMFCS level—initial	GMFCS level—final
1	2	3 + 3	4 + 0	V	V[b]
2[a]	2	6 + 6	7 + 4	V	IV[b]
3	2	3 + 3	3 + 10	V	V[b]
4	2	1 + 9	2 + 4	V	V
5	2	2 + 3	2 + 9	V	V
6	2	1 + 3	1 + 9	V	III
7[a]	3	1 + 1	1 + 8	V	IV
8	3	2 + 5	3 + 1	IV	IV[b]
9[a]	3	3 + 0	3 + 8	IV	II
10[a]	3	3 + 6	4 + 3	IV	III
11	3	4 + 0	4 + 8	IV	IV
12	3	4 + 3	5 + 0	V	V [b]
13	3	5 + 7	6 + 2	V	V[b]
14	3	6 + 5	6 + 11	IV	IV[b]
15[a]	3	9 + 6	10 + 2	IV	II
16	3	6 + 2	6 + 9	IV	IV[b]
17[a]	3	6 + 2	7 + 6	IV	III
18	3	4 + 6	5 + 4	IV	IV
19	4	1 + 6	2 + 6	V	V
20	4	7 + 0	8 + 1	V	V
21	4	4 + 1	5 + 4	V	V
22	4	7 + 1	8 + 2	V	V
23[a]	4	6 + 8	7 + 6	V	IV
24[a]	4	5 + 3	6 + 8	V	IV
25[a]	4	3 + 7	5 + 0	V	III
26[a]	4	2 + 0	3 + 5	V	IV
27	4	2 + 9	4 + 3	V	V
28[a]	4	6 + 5	7 + 0	V	IV
29	4	7 + 3	8 + 6	V	V[b]
30	4	5 + 1	6 + 2	IV	IV[b]
31[a]	5	2 + 0	2 + 9	V	IV
32[a]	5	2 + 4	4 + 0	IV	III
33	5	7 + 5	8 + 9	V	V
34[a]	5	7 + 5	9 + 2	IV	III
35[a]	5	6 + 4	8 + 2	V	II
36[a]	6	7 + 3	8 + 3	V	IV
37[a]	6	1 + 8	3 + 4	V	III
38[a]	6	4 + 0	6 + 10	IV	II

General headings for each GMFCS level: level I, walks without limitations; level II, walks with limitations; level III, walks using a handheld mobility device; level IV, self-mobility with limitations, and may use powered mobility; level V, transported in a manual wheelchair.
[a]Responders ($n = 18$).
[b]Patients in whom CP was accompanied by epilepsy.

patients with milder forms of the disease (hemiplegia and diplegia). Adverse reactions (fever, nausea, vomiting and hemoglobinuria) were observed in five patients and ascribed to cryoprotectant toxicity and/or hemolysis caused by thawing.

The feasibility of allogeneic UCB cell infusions into patients with non-hematopoietic degenerative conditions was evaluated in the study conducted by Yang *et al.* [38]. One hundred fourteen patients with paraplegia, ataxia, multiple or amyotrophic lateral sclerosis, among other conditions, received multiple (up to 4–5) intrathecal injections combined with intravenous infusion of allogeneic HLA-unmatched UCB mononuclear cells ($1–3 \times 10^7$ cells per treatment). Adverse reactions (headache, fever, waist pain, vomiting, etc.) occurred in 38 (6.42%) of 592 injections and were probably related to the effects of the cryoprotectant and/or the intrathecal route of administration. No serious adverse hematological, biochemical or immunological effects were reported. Later, researchers reported significant improvements (>50%, Berg Balance Scale) after 4–6 combined intrathecal/intravenous infusions of allogeneic UCB cells in 13 of 30 patients with hereditary ataxia; in 17 other patients, the effect was smaller (5–49%) [39].

In a recent publication (results of the NCT01193660 trial, clinicaltrials.gov), Min *et al.* assessed the safety and efficacy of allogeneic (4–5 of six HLA-antigen matched) UCB cell transplantation with concomitant administration of recombinant

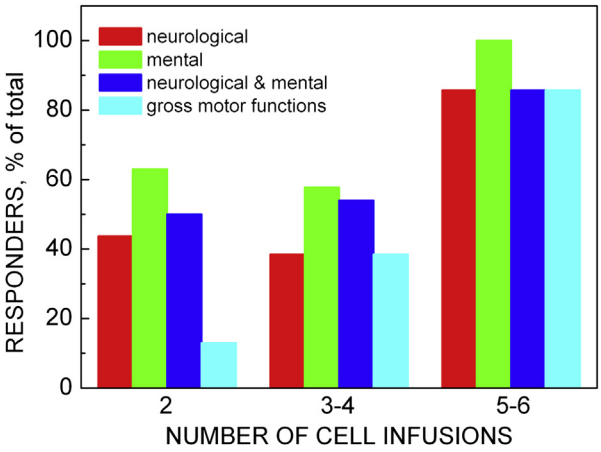

Figure 4. The dependence of cell therapy efficacy on the number of UCB cell infusions. Data are presented as the relative number of responders in each cohort.

human erythropoietin (rhEPO) in 105 children with CP [40]. According to the study results, cell infusion potentiated with rhEPO significantly ameliorated motor and cognitive impairment compared with pure rhEPO or a UCB placebo. Unfortunately, the absence of a UCB-alone group did not allow for the described effects of treatment to be attributed the UCB cells. Furthermore, many adverse events were clearly associated with rhEPO administration and/or immunosuppression with cyclosporine.

Therefore, the occurrence of adverse events and/or other unwanted reactions to UCB cell infusion is likely to be associated primarily with the peculiarities of preparation of therapeutic cell products and the route of administration but not with the UCB source (autologous or allogeneic), which is particularly important in the treatment of young children.

Irrespective of a considerable body of clinical evidence confirming the effectiveness of UCB cell therapy for patients with cerebral and spinal injuries of various geneses, the mechanisms underlying their effects remain obscure [19,41]. By producing neurotrophic factors, these cells may contribute to the regeneration of the central nervous system as regulators of neuronal and glial activity [11]. Both UCB cells and cells derived as a result of their differentiation *in vitro* produce various neurotrophic factors, such as brain-derived neurotrophic factor, glial-derived neurotrophic factor and neurotrophins 3 and 4-5 [9,10]. Additionally, UCB cells secrete an array of biologically active compounds (GRO-α, MIP-1α, MCP-1, MCP-3, RANTES, SDF-1, G-CSF, GM-CSF, interleukins [IL]-6 and -8) with neuroprotective, immunomodulating, anti-apoptotic and anti-inflammatory activities [11]. Presumably, paracrine regulation, including neuroprotection and stimulation of neurogenesis in the brain, is the major mechanism responsible for the therapeutic effect of systemic administration of UCB cells [12,42].

In this context, the finding that the cytokine levels in CP patients' blood may depend on the donor cell source (autologous or allogeneic) is particularly interesting. Therefore, Sang-Hun Bae *et al.* [43] have demonstrated that the levels of pro-inflammatory factors (IL-1α, IL-6, tumor necrosis factor-β and RANTES) are significantly decreased in response to allogeneic UCB cell infusion, whereas the changes in motor improvement and social behavior were considerably greater compared with the autologous treatment group.

Conclusions

This study has demonstrated the safety and efficacy of allogeneic AB0/Rh-identical UCB cells in young patients suffering from CP. It should be noted that the most pronounced therapeutic effect was achieved after multiple infusions of these cells. The results support the expectations of several experts who have recently proposed [44] that "an allogeneic 'off-the-shelf' product … would make UCB cell–based intervention available to all infants and children in need of this therapy."

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