Review

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Future perspectives of cell therapy for neonatal hypoxic–ischemic encephalopathy

Makoto Nabetani[1,2], Haruo Shintaku[2] and Takashi Hamazaki[2]

Neonatal ischemic brain injury causes permanent motor-deficit cerebral palsy. Hypoxic–ischemic encephalopathy (HIE) is a very serious condition that can result in death and disability. In 1997, we reported that irreversible neuronal cell damage is induced by the elevation of intracellular Ca ion concentration that has occurred in sequence after excess accumulation of the excitatory neurotransmitter glutamate during ischemia. We also reported that hypothermia was effective in treating ischemic brain damage in rats by suppressing energy loss and raising intracellular Ca ion concentration. Following the 2010 revised International Liaison Committee on Resuscitation guideline, our group developed the *Guideline for the treatment of Hypothermia* in Japan, and we started online case registry in January 2012. However, therapeutic hypothermia must be initiated within the first 6 h after birth. By contrast, cell therapy may have a much longer therapeutic time window because it might reduce apoptosis/oxidative stress and enhance the regenerative process. In 2014, we administered autologous umbilical cord blood stem cell (UCBC) therapy for neonatal HIE, for the first time in Japan. We enrolled five full-term newborns with moderate-to-severe HIE. Our autologous UCBC therapy is leading to new protocols for the prevention of ischemic brain damage.

Although the neonatal mortality rate has decreased considerably over the past several decades worldwide, a vast difference among many countries still exists (1). In Japan there has been a significant decrease in the neonatal mortality rate in the past 30 years—from 2.7/100,000 to 1.0/100,000 births, similar to that in other countries with a sophisticated medical system. However, the prevalence of cerebral palsy (CP) due to preterm and term ischemic brain injury has remained at the same rate over several decades, even in highly developed countries (2–4). Neonatal brain injury in full-term infants is caused mainly by neonatal hypoxic–ischemic encephalopathy (HIE), congenital anomaly, and cerebral infarction, whereas neonatal brain injury in preterm infants is caused mainly by periventricular leukomalacia (PVL) and intraventricular hemorrhage (5). HIE in term infants is a particularly serious condition; it occurs in an estimated 0.5–2/1,000 live births and results in death and disability (6–8). In the past, more than half of moderate-to-severe cases of neonatal HIE resulted in permanent motor-deficit CP, and CP was often accompanied by other severe complications, such as hearing loss, visual disturbance, epilepsy, hydrocephalus, intellectual disability, and behavioral problems (9). In recent years, however, therapeutic brain hypothermia has been established as the first effective therapy for neonates with HIE (10–12). Furthermore, cell therapies such as umbilical cord blood stem cells (UCBCs), bone marrow (BM) stem cells, and umbilical cord/BM–derived mesenchymal stem cells (BM-MSCs) are being incorporated into new protocols for protection against ischemic brain damage.

MECHANISM OF NEONATAL HIE

In 1969, Olney (13,14) discovered excitotoxicity and demonstrated that at least some of the neural cell death caused by hypoxia–ischemia was mediated by excess production of the excitatory neurotransmitter glutamate. In contrast to the experience with adult hypoxic–ischemic insults, neonatologists found that some infants who had recovered smoothly from severe asphyxia subsequently deteriorated rapidly and died a few days later. No effective therapy was available for such brain damage for several decades. In the 1980s, researchers reported the phenomenon of delayed neuronal death (15,16). In the 1990s, Osmund Reynolds's group confirmed the phenomenon of delayed neuronal death of newborns after hypoxic ischemic insults, called "secondary energy failure," using a sophisticated phosphorus magnetic resonance spectroscopy approach to replicate the complicated process in piglets and rat pups (17–20).

In 1984, Olney and co-workers (21) shifted the paradigm, proposing that hypoxic–ischemic damage can be treated by blocking *N*-methyl-D-aspartate and suggesting that it can be blocked pharmacologically to provide good protection against neonatal hypoxic–ischemic brain damage. Regrettably, the *N*-methyl-D-aspartate receptor blocker and other drugs, such as magnesium sulfate and calcium channel antagonists, were not effective clinically (22,23). In 1997, we reported that

[1]Department of Pediatrics, Yodogawa Christian Hospital, Osaka, Japan; [2]Department of Pediatrics, Faculty of Medicine, Osaka City University 1-4-3 Asahi-cho, Abeno-ku, Osaka, Japan. Correspondence: Makoto Nabetani (nabe35001@gmail.com)

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irreversible neuronal cell damage was triggered by an elevation of intracellular Ca ion concentration subsequent to excessive accumulation of the excitatory neurotransmitter glutamate in immature and mature rats during ischemia and glucose deprivation (24). The crucial role of free radicals to result in irreversible cell damage was generated in considerable part of Ca^{2+}-activated processes (25–30). However, it is clinically impossible to pinpoint the timing of these mechanisms and identify which mechanism determines the severity of HIE in human neonates (31,32).

In 1989 Busto *et al.* (33) showed that mild hypothermia after hypoxia–ischemia insult in adult rats reduced the release of neurotransmitters and had a protective effect on hippocampal neuronal injury. In 1996, Thoresen *et al.* (34) and Sirimanne *et al.* (35)reproduced the protective effect of hypothermia against brain injury in neonatal rats. We reported that hypothermia therapy was an effective treatment for hypoxic or ischemic brain damage in rats by suppressing energy loss and elevation of intracellular Ca ion concentration (36)

MECHANISM OF STEM CELL THERAPY AS A REGENERATIVE TREATMENT FOR NEONATAL HIE

Recent experimental studies in animal models have indicated that various mechanisms of action are involved in the process by which UCBCs protect the brain from hypoxic–ischemic damage. They may also be expected to enhance recovery from brain damage. The brain damage process is divided into five stages: (1) energy depletion, (2) inflammation, (3) excitotoxity, (4) oxidative stress, and (5) apoptosis (**Figure 1**) (27–29). Cord blood stem cell therapy has been suggested to provide a protective effect mainly on (2) inflammation, (4) apoptosis, and (5) oxidative stress, as well as to enhance regeneration.

Immunomodulation/Anti-Inflammatory Action

It is not yet known which component of cord blood is most efficacious for treating brain injury-mediated inflammation. Specific cell populations found in cord blood and tissue, such as MSCs and endothelial progenitor cells, have demonstrated potential utility for mitigating the inflammatory process induced by brain injury. MSCs have strong immunomodulatory effects, protecting against global and local neuroinflammatory cascades triggered by hypoxic–ischemic events (37–39). Some reports suggest that UCBC administration also reduces white matter injury after hypoxic–ischemic insult, via a combination of anti-inflammatory and other actions (40).

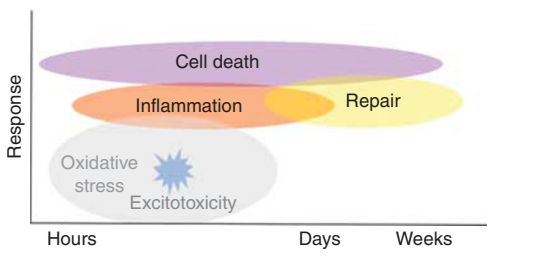


Figure 1. Neonatal brain injury in hypoxic–ischemic encephalopathy.

Reduction of Apoptosis and Oxidative Stress

Hattori reported that a single intraperitoneal injection of UCBC-derived mononuclear cells 6 h after an ischemic insult was associated with a transient reduction in the number of apoptosis and oxidative stress marker–positive cells, but it did not induce long-term morphological or functional protection (41–43).

Enhancement of the Regenerative Process by the Secretion of Various Cytokines

Human $CD34^+$ cells have been shown to secrete various growth factors such as brain-derived neurotrophic factor, glial cell line–derived neurotrophic factor, vascular endothelial growth factor, and numerous angiogenic factors, including hepatocyte growth factor and insulin-like growth factor-1 (44–47).

Enhancement of the Regenerative Process by Angiogenesis for Better Circulation of the Brain

In 2004, Taguchi *et al.* (48,49) reported that after stroke $CD34^+$ cells provide a favorable environment for neuronal regeneration, suggesting an essential role of $CD34^+$ cells in directly or indirectly promoting an environment conducive to neovascularization of the ischemic brain. It is evident that circulating endothelial progenitor cells in $CD34^+$ cell populations enriched in cord blood have the capacity to participate in the neovascularization of ischemic tissue in neonates (50,51). Endothelial progenitor cells have angiogenic and vascular reparative capabilities that make them ideal for neurovascular repair (52,53). Such a rich vascular environment, along with the generation of other nurturing neuronal mediators from $CD34^+$ cells, such as vascular endothelial growth factor, epidermal growth factor 2, and insulin-like growth factor 1-1 (refs (54,55)), enhances subsequent neuronal regeneration. Endogenous neurogenesis is accelerated by neuronal progenitors to the damaged area, followed by their maturation and survival when $CD34^+$ cells continue to stimulate the formation of vascular channels (47,56).

Enhancement of the regenerative process by neurogenesis

Neural stem/progenitor cells have been shown to participate in the regenerative response to perinatal hypoxia–ischemia (57). One article reported that hematopoietic stem cells could differentiate into nonlymphohematopoietic cells such as neurons or microglia or could stimulate neurogenesis (58). However, it is uncertain whether this is significantly effective for neonates with HIE (59–63).

FRAMEWORK OF PROTECTIVE THERAPY FOR NEONATAL HIE IN JAPAN

In the early twenty-first century, therapeutic hypothermia (TH) was used for newborns with HIE. Three large-scale randomized controlled trials were performed and proved the

feasibility and efficacy of TH in 2005 (ref. (10–12)). Furthermore, the 2010 revised International Liaison Committee on Resuscitation guideline by Perlman and co-workers (64) stated that infants born at or near term with evolving moderate-to-severe HIE should be offered TH. In Japan, TH was started in 1999. However, various empirical approaches prevailed until 2010, and the number of centers capable of providing standard cooling was insufficient at that time (**Figure 2**). Therefore, in August 2010 we conducted a Primary Nationwide Practice Survey on TH to investigate the state of practice in Japan. Questionnaires were sent to all registered level II/III neonatal intensive care units, and responses were obtained from 203 of 242 units (83.9%) (65). Only 89 facilities (43.8%) said they provide TH. The number of infants with HIE in 2009 in the level II/III neonatal intensive care units was 486. The number of cooled infants in 2009 was 234.

These results suggest that about half of newborns with HIE might not have received any benefit from TH in 2010. Therefore, we established the Neonatal Hypothermia Task Force, Japan and developed the *Guideline for the treatment of Hypothermia* in Japan based on randomized controlled trials in 2011 (ref. (66)). Then, we held several workshops and consensus meetings to formulate clinical recommendations, which were followed by the publication of practical textbooks and large-scale education seminars. We started an online case registry in January 2012. Findings from the follow-up survey in January 2013 were compared with the results from the primary survey (response rate: 89.1%). The number of cooling centers increased from 89 to 135. Twelve of 47 prefectures had no cooling centers in 2010, whereas all 47 prefectures had at least one in 2013. In cooling centers, adherence to the standard cooling protocols and the use of servo-controlled cooling devices improved from 20.7% to 94.7% and from 79.8% to 98.5%, respectively. As of December 2016, 900 cases ($>$200 cases/year) and 167 cooling units have been registered. A rapid improvement in the national provision of evidence-based TH has been achieved. Our strong interventions in accordance with the international consensus guidelines might be effective in shifting empirical approaches to evidence-based practice (67).

To examine the clinical use of TH, we analyzed the data collected during the first 3 years (2012–2014) of the Baby Cooling Registry of Japan (67). Of 485 cooled neonates, 96.5% were at $\geq$36 weeks gestation; 99.4% weighed $\geq$1,800 g. In addition, 96.7% required resuscitation for $>$10 min. Stage II and III encephalopathy was evident in 61.1% and 27.2%, respectively. The mortality rate was 2.7%; 90.7% were discharged home. Apgar scores and severity of acidosis/encephalopathy did not change over time: 1 (1 min), 4 (5 min), and 5 (10 min), and pH (6,68). The time to reach the target temperature was shorter in 2014 than in 2012. Mortality, duration of mechanical ventilation, and requirement for tube feeding at discharge remained unchanged. The mortality rate in our cohort (2.7%) was considerably lower than that reported in previous studies (CoolCap (33%), NICHD (24%), and UK TOBY (26%)), despite the use of similar inclusion criteria (10–12). A more recent clinical study showed a lower short-term mortality rate (7%) in neonates who were randomized to whole-body cooling to 33.5 °C for 72 h (69). This may be attributable to the difference in attitudes regarding withdrawing life support for newborns with very severe brain damage. Global comparative studies are needed to illuminate the factors associated with short- and long-term outcomes of cooled neonates. However, TH has the following restrictions:

(1) Its window of opportunity is ~6 h after birth, and earlier treatment after birth is more effective.
(2) The number needed to treat is ~9, and so many babies still die or survive with disability.

Furthermore, a recent randomized controlled trial demonstrated that longer cooling and/or cooling at lower temperatures, compared with hypothermia at 33.5 °C for 72 h, did not reduce neonatal intensive care unit death among full-term neonates with moderate or severe HIE (69).

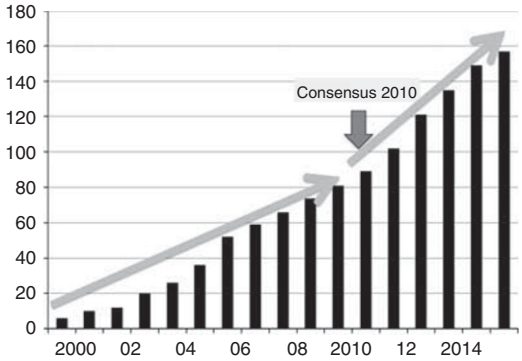


Figure 2. The number of cooling centers in Japan for treatment of infants with hypoxic–ischemic encephalopathy.

CLINICAL APPLICATION OF UCBCS THERAPY FOR ISCHEMIC BRAIN INJURY

In 1982, Nakahata and Ogawa (70) reported that UCB (Umbilical Cord Blood) contains rich stem cells such as hematopoietic and MSCs. CD34 surface antigen has been widely used as a marker of hematopoietic stem and endothelial progenitor cells. UCB contains ~0.3–2% $CD34^+$ cells, whereas the peripheral blood of adult humans contains $<$0.01% $CD34^+$ cells (71–75). The therapeutic effects of UCB have previously been shown in hematological diseases, such as leukemia, Fanconi's anemia, and aplastic anemia, replacing the use of hematopoietic stem cells over the past few decades (76–78). However, in recent years, UCB has been identified as a source of endothelial stem/progenitor cells and as having an effect on various intractable diseases, including CP, diabetes mellitus, and cardiac, vascular, and hepatic diseases. Various stem cell types are possible sources of cell therapy for clinical applications, especially for neurological diseases (79,80). Below we highlight the potential therapeutic effects of cell-

based therapies, particularly autologous cord blood therapy for ischemic disease, which has been progressing markedly in the past few decades.

In 2006, Meier *et al.* (81) reported the effectiveness of intraperitoneal infusion of UCBCs for rats with neonatal hypoxia. Kurtzberg and co-workers (82), at the Duke University in the United States, is conducting a phase II study for CP, using autologous UCBCs. In 2011, Cox *et al.* (82) reported a feasibility study showing that autologous BM mononuclear cells were logistically feasible and safe to prescribe intravenously for children suffering from traumatic brain injury. Ten children aged 5 to 14 years who had postresuscitation Glasgow Coma Scale scores of 5 to 8 were treated with 6×10 autologous BM mononuclear cells per kg body weight delivered intravenously within 48 h of traumatic brain injury. All the patients survived. Conventional magnetic resonance imaging comparing gray matter, white matter, and cerebral spinal fluid volumes showed no reduction from 1 to 6 months after injury. The Dichotomized Glasgow Outcome Score at 6 months showed 70% with good outcomes and 30% with moderate-to-severe disability.

Wang *et al.* (83) reported a clinical study using BM-MSCs in CP patients in 2013. They suggested that autologous BM-MSC transplantation may be a feasible, safe, and effective therapy for patients with CP. The treatment improved the development of motor function in children with CP (84). After providing informed consent, 52 patients with CP who met the study criteria received BM-MSC transplantation. Gross motor function was assessed at baseline (before transplantation) and at 1 month, 6 months, and 18 months after transplantation. The gross motor function score domains A, B, C, and D and the total scores in participants increased at 1 month, 6 months, and 18 months after transplantation compared with the baseline value ($P < 0.01$). The scores of domain E also increased at 6 and 18 months after transplantation. The gross motor function scores increased significantly after cell transplantation.

In 2015, Sharma *et al.* (84) conducted an open-label, nonrandomized study in 40 CP patients, with the aim of evaluating the benefit of cellular therapy in combination with rehabilitation. These patients received autologous BM mononuclear cells intrathecally. The follow-up was carried out at 1 week, 3 months, and 6 months after the intervention. Overall, at 6 months, 95% of patients showed improvements. The study population was further divided into diplegic, quadriplegic, and miscellaneous groups. On statistical analysis, a significant association was established between cell therapy and symptomatic improvement in diplegic and quadriplegic CP. Positron emission tomography-computed tomography scans performed in six patients showed metabolic improvements in areas of the brain correlating with clinical improvement. The results of this study demonstrate that cellular therapy may accelerate development, reduce disability, and improve the quality of life of patients with CP (84).

It has also been reported that concomitant administration of allogeneic UCBCs and recombinant human erythropoietin may boost the efficacy of UCBCs and ameliorate motor and cognitive dysfunction in children with CP undergoing active rehabilitation, accompanied by structural and metabolic changes in the brain (85). In total, 96 subjects completed the study. Compared with the recombinant human erythropoietin ($n = 33$) and control ($n = 32$) groups, the recombinant human erythropoietin and allogeneic UCBCs ($n = 31$) group had significantly higher scores on the gross motor performance measure and Bayley scales of infant development-II mental and motor scales at 6 months. Diffusion tensor images revealed significant correlations between the gross motor performance measure increment and changes in fractional anisotropy in the recombinant human erythropoietin and allogeneic UCBCs group. ^{18}F-fluorodeoxyglucose positron emission tomography-computed tomography showed different activation and deactivation patterns between the three groups.

One hundred and eighty patients with diplegia and quadriplegia after trauma underwent subarachnoid placement of stem cells between December 2005 and October 2007 in India. In 102 (56.6%) patients, side effects were observed (headache, low-grade fever, and meningism), which resolved with symptomatic treatment within 24 h. It was effective in 32% of patients, with neither short- nor long-term adverse effects. In long-term follow-up, functional indices improved in 57 (31.67%) patients: 54 patients with traumatic paraplegia/quadriplegia, 2 with CP, and 1 with viral encephalitis (86).

Recently, Mancías-Guerra *et al.* (87) reported an open-label phase I trial to investigate the safety and tolerability of intrathecal delivery of autologous BM nucleated cells in children with CP. Eighteen pediatric patients with CP were studied to assess the safety of autologous BM–derived total nucleated cell intrathecal and intravenous injection after stimulation with granulocyte colony-stimulating factor. An overall 4.7-month increase in developmental age according to the Battelle Developmental Inventory, including all areas of evaluation, was observed ($\pm$ SD 2.63). No magnetic resonance imaging changes at 6 months of follow-up were found. Subarachnoid placement of autologous BM–derived total nucleated cells in children with CP is a safe procedure.

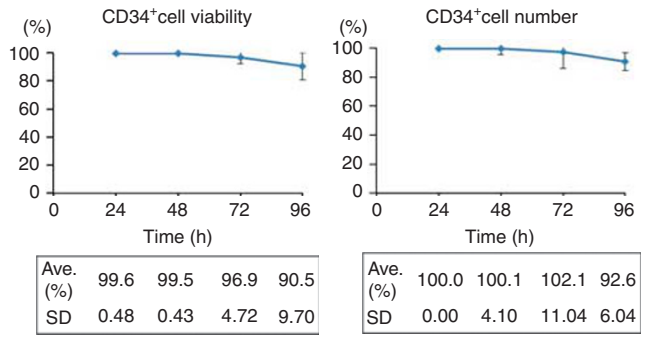
Compared with BM–derived cells, UCBCs are readily available if adequately harvested and stored. Cell therapy using UCBCs has been expanding for novel applications. In a review article, Rizk *et al.* (88) reported that the most common indication for UCB therapy is neurological diseases (25 studies), including CP (12 studies). Other indications include diabetes mellitus (9 studies), cardiac and vascular diseases (7 studies), and hepatic diseases (4 studies). Most of the studies used total nucleated cells, mononuclear cells, or $CD34^+$ cells (31 studies); 20 studies used cord blood–derived MSCs. Forty-six studies described cellular products obtained from allogeneic sources, and 11 studies used autologous products. Rizk *et al.* (88) identified three indications for which multiple prospective controlled studies have been published (4 of 4

reported clinical benefit in CP, 1 of 3 reported benefit for cirrhosis, and 1 of 3 reported biochemical response in type 1 diabetes).

FUTURE PERSPECTIVES OF CELL THERAPIES FOR NEONATAL HIE

Feasibility of Autologous Cord Blood Therapy

TH has some limitations in that it must be initiated within the first 6 h after birth. Recently, a randomized controlled trial of whole-body hypothermia for 72 h in preterm infants with a gestational age of 33–35 weeks was started, but TH is under investigation for premature babies <33 weeks. By contrast, cell therapy may have much longer therapeutic time windows (89) and might show effectiveness for ischemic brain damage of term, near-term, and premature newborns, including periventricular leukomalacia, intraventricular hemorrhage, and HIE. Many clinical trials are currently underway to investigate the efficacy of stem cells to treat patients with perinatal ischemic brain damage and CP. Stem cells obtained from umbilical cord tissue and cord blood, normally discarded after birth, are emerging as a safe and potentially effective therapy. Among these different cell therapy strategies, intravenous administration of autologous UCBC therapy could be the safest and most feasible because UCB has been used for hematopoietic stem cell transplantation in patients with hematological diseases for several decades (90). Furthermore, UCB contains several types of stem cells such as hematopoietic stem cells, endothelial progenitor cells, and MSCs (91,92). Collection of UCB is noninvasive and autologous, and the use of UCBCs is associated with fewer ethical issues compared with allogenic or cultured stem cells, embryonic stem cells, and induced pluripotent stem cells because autologous UCB carries no possibility of tumorigenicity or rejection and does not require a complicated culture process. Collection, separation and storage of UCBCs have to be regulated carefully because screening for infection is required. We propose that autologous UCBCs, in combination with TH, could be the optimal therapy for newborns with HIE. We also suggest that autologous UCBC therapy might be the most feasible treatment for premature newborns with periventricular leukomalacia or intraventricular hemorrhage who were born at 24–33 weeks of age.



Ave. (%)	99.6	99.5	96.9	90.5
SD	0.48	0.43	4.72	9.70

Ave. (%)	100.0	100.1	102.1	92.6
SD	0.00	4.10	11.04	6.04

Figure 3. Viability of $CD34^+$ cells after separation.

Experience with Autologous UCBC Therapy in Japan

Cotten *et al.* (93) showed the feasibility and potential effectiveness of autologous UCBC therapy for neonatal HIE. In 2014, we established the Neonatal Encephalopathy Consortium, Japan research group for autologous UCBC therapy for neonatal HIE and started using autologous UCBC therapy for neonatal HIE. This is a pilot study for testing the feasibility and safety of UCBC therapy in infants with neonatal HIE; the study is an open-label, single-group assignment. $CD34^+$ cells decrease rapidly in the neonatal peripheral blood immediately after birth and tend to reach the basic level within the first 48 h after delivery (94). Before proceeding with the human trial, we investigated the viability and numbers of $CD34^+$ cells in the UCB after automated mononuclear cell separation. UCB was collected from mothers who had given prior written informed consent for the collection. The aseptically collected UCB was processed using Sepax (Biosafe, Eysins, Switzerland) to reduce volume and red blood cells for three separate infusions. Without cryopreservation, the total number and viability of $CD34^+$ cells in the processed UCB remained unchanged over 72 h (**Figure 3**). The potassium concentration was also stable after 72 h, to a median of 5.8 mEq/l (1.7–11.6). Because Sepax requires at least 40 ml volume without any clotting for normal processing, we had to set exclusion criteria for infants whose collected UCB was < 40 ml or had massive clotting. In addition, the enrollment criteria for our ongoing autologous UCBC study is the same as the inclusion/exclusion criteria for TH in Japan (68). If a neonate is born with signs and symptoms of moderate-to-severe encephalopathy and meets the criteria for TH, the neonate is considered for entry to this clinical study. The estimated enrollment is six cases. To ensure that UCB is properly collected without contamination, we exclude outborn infants from the trial. If an infant is born with severe asphyxia, the UCB is collected directly after birth from an umbilical cord vein, with special care to avoid contamination. We obtain parental consent before collecting UCB. UCB is volume and red blood cell reduced by centrifugation in a closed system using an automated machine (Sepax). The volume- and red blood cell–reduced UCB contains numerous types of nucleated cells, including a variety of stem cells, such as $CD34^+$ hematopoietic stem/endothelial progenitor cells. The processed UCB is divided into three doses and stored at 4 °C until use. The cell dose is not adjusted. The total amount of UCB collected is used after the above-mentioned simple centrifugation. The estimated doses administered would be $\sim 6 \times 10^8$ cells per newborn.

If the total amount of UCB is <40 ml, the newborn will not be enrolled in the trial because the automated UCB process may not be reliable if the volume to be processed is <40 ml. We examined the quality of the processed and noncryopreserved UCB using UCB collected from volunteers before commencement of this trial. At 72 h after the processing, there was no growth of bacteria or increase in potassium, and cell viability was well maintained. We obtain written informed consent from the parents twice: first, when we judge that the

Table 1. Patient profiles in USBC pilot study

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Gestational age (week per date)	38w1d	40w0d	41w4d	39w5d	38w5d
Birth weight (g)	2,436	2,507	3,024	4,086	2,723
Apgar score (1 min/5 min)	2/5	0/0	2/2	5/6	2/7
Complications at delivery	Abruption	Cord prolapse	Mother CPA	—	—
pH (cord blood)	7.2	6.9	7.1	7.2	7.1
Base deficit (cord blood)	7.4	20	9	5.1	10
Sarnat staging	II	III	II	II	II
Thompson	15	12	9	12	15

CPA, cardiopulmonary arrest; UCBC; umbilical cord blood stem cell.

newborn with HIE meets the entry criteria after the initial assessment, which is normally a few hours after birth; second, before the first administration of UCBC treatment for the newborn.

Autologous volume-reduced cord blood cells are administered intravenously at 12–24, 36–48, and 60–72 h after birth. Circulatory and respiratory status is closely monitored during and after the cell treatment. The primary outcome measure is the rate of adverse events. The combined rate of three adverse events at 30 days of age—death, continuous respiratory support, and continuous use of vasopressor—will be compared between the neonates receiving cell therapy and those with conventional therapy including hypothermia. The secondary outcome measure is efficacy. Neuroimaging at 12 months of age and neurodevelopmental function measured with Bayley III at 18 months of age will be compared between the cell recipients and neonates with conventional therapy. The infants will be followed for safety and neurodevelopmental outcome up to 10 years of age.

We studied five patients who underwent autologous UCBC therapy in December 2014–December 2016. We did not detect any significant adverse effects of the treatment. We present the profiles of five cases in **Table 1**. We enrolled one patient with severe HIE and four patients with moderate HIE. The patient with severe HIE and the one with moderate HIE showed abnormality on brain magnetic resonance imaging, but all five survived up to 1 year. Additional randomized clinical trials are needed to prove the effectiveness of autologous UCBC therapy over the TH-only treatment.

CONCLUSION

Cell therapies such as autologous UCBCs and BM stem cells, umbilical cord/BM-MSCs, and other stem cell therapies are leading to new protocols for the prevention of ischemic brain damage. Further preclinical studies are expected to optimize the treatment protocol, and multicenter clinical trials are needed to prove safety and efficacy.

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