

Prevention and treatment of cerebral palsy with cord blood stem cells and cord-derived mesenchymal stem cells

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Prevention and treatment of cerebral palsy (CP) by regenerative medicine:

Perinatal asphyxia is a well-known medical condition that can lead to CP. Several etiologies are involved in this process, but the primary cause is hypoxic-ischemic encephalopathy (HIE) that is characterized by reduced blood flow and oxygen supply to the baby's brain. CP is one of the possible consequences of this neurologic injury. Once developed, CP is difficult to treat, and the role of rehabilitation in functional recovery is still limited. The Neonatal Resuscitation Program is one of the various approaches that have been tried to date. The program has been providing training courses since 2006 for midwives and nurses in addition to physicians, and we saw a marked reduction in mortality associated with birth asphyxia just after a year of the program. However, further efforts are required to achieve 0% mortality.

Since its first introduction in the late 1990s, therapeutic hypothermia (TH) has become a widely accepted intervention. With official guidelines already established, it is now a standard of care in medium- to large-scale perinatal centers. The decreasing mortality rate in the five years after the guideline publication has proven the efficacy of TH. Still, more than 50% of infants develop CP as a sequela. Since TH alone is not satisfactory, a clinical study of autologous cord blood stem cells (CBSCs) transplantation has been initiated to examine its efficacy.

In severe birth asphyxia, hypoxia-ischemia damages the white matter, the gray matter and the central part of the brain called the basal ganglia. At 10 days to 1 month after birth, the basal ganglia appear white on MRI images, even though patients were treated with hypothermia. This is because head cooling is not deep enough to reach the central part of the brain (the basal ganglia), as it cools the brain from the surface. This is the reason why whole body cooling is now the most widely used modality. Published studies have suggested a possible mechanism of brain damage in HIE. First, oxidative stress, inflammation and energy failure occur several hours after the initial ischemic insult. In the following several hours to days, cellular inflammation leads to necrosis and cell death (apoptosis). Over the next several days to weeks, the body attempts to repair the damage. Ultimately, these processes lead to recovery, but at the same time damage continues to accumulate. Hypothermia suppresses inflammation by reducing the production of excitatory neurotransmitters, intracellular calcium and nitric oxide and free radicals involved in these processes. Cooling has been shown to act on both necrotic and apoptotic pathways.

Can TH reduce the damage itself and optimize the recovery? Unfortunately, it is considered not enough. This is where stem cell therapy has

a potential role. Stem cells can secrete potent combinations of trophic factors that modulate the molecular composition of the environment to evoke responses from resident cells (Branjak and McDevitt, 2010). Regenerative medicine using stem cells, in particular, the CBSCs provide protection from inflammation, apoptosis, oxidative stress, enhance regeneration, immune regulation, and anti-inflammatory effect (Qin et al., 2019). Among several donor sources, some researchers are focusing on autologous CBSCs to advance their clinical research, because there is no need to culture and it can be administered immediately and safely. At Duke University, autologous CBSCs therapy was started in the treatment of HIE as a prophylaxis for CP, but then it has also been used as a treatment for CP (Boruczowski et al., 2019).

In vitro and animal studies: *In vitro* experiments have demonstrated the expression of neural and normal cell markers in human nucleated cord blood cells and the expression of oligodendroglial and astrocytic features in CD34⁺ and other CBSCs. In regenerative medicine cell replacement is an intuitive concept in which infused stem cells are considered to replace damaged neuronal cells. However, the transplanted human mononuclear cells were traced, and after 14 days, they were shown to be engrafted but not differentiated into astrocytes. After all, the differentiation of nucleated human CBSCs into neurons shown in the *in vitro* experimental system was not shown in the *in vivo* experimental system.

In vivo studies using a neonatal rat model of HIE have shown that transplantation of human umbilical cord blood CD34⁺ cells can ameliorate the neural functional defect and reduce apoptosis and promote nerve and vascular regeneration in the rat brain after hypoxic ischemic injury (Yu et al., 2019). Although following brain injury, neuronal cell death, edema, inflammation and apoptosis occur due to post-injury excitotoxicity, a comparison of the injured hemisphere and the contralateral uninjured hemisphere at 7 days after the insult revealed activated microglia and astrocytes in the lesioned hemisphere, and CBSCs transplantation resulted in a reduction in activated microglia in the lesioned hemisphere (Pimentel-Coelho et al., 2010). Stem cells are expected to improve the outcome by not developing into neuronal cells but promoting intrinsic repair mechanisms, which reduce the brain damage and facilitate the repair process.

History of CBSCs: Since CBSCs were discovered in 1982, these cells have been used for the treatment of various diseases including Fanconi's anemia and leukemia. They have also been used for inherited metabolic diseases since the turn of the century. Krabbe disease is one such example. Krabbe disease is a hereditary and

progressive demyelinating disease characterized by accumulation of lipid metabolites in the brain leading to death at 2 to 3 years of age. In a subsequent study, the CBSCs from unrelated donors was used for the treatment of Krabbe disease (Escobar et al., 2005). Eleven asymptomatic neonates and 14 symptomatic infants underwent the transplantation of allogeneic cord blood, and their survival was compared to that of 190 untreated patients. The authors found that all patients in the untreated group died before 96 months of age, while 40% of the treated symptomatic patients survived after this age. The survival rate was 100% in treated asymptomatic infants.

In 2009, it was reported that CBSCs transplantation in an inherited metabolic disease holds promise as a new treatment. Under these circumstances, basic research on CBSCs for the treatment of central nervous system disorders due to hypoxia, ischemia, etc., that is, research on regenerative medicine of the brain using cord blood stem cells has been actively conducted.

Approach to central nervous system deficits using cord blood cells:

These studies all support the possible efficacy of CBSCs therapy in other central nervous system diseases and led to CBSCs transplantation in patients with CP and HIE in the 2010s. Cotten et al. (2014) led the first clinical trials for CP and HIE. Although the mortality at 15 months was not significantly different, autologous CBSCs in HIE patients resulted in a significantly higher survival with Bayley III scores ≥ 85 (Cotten et al., 2014). Cotten et al. (2014) used a closed cell separation system (i.e., no exposure to air contaminants) called Sepax to isolate autologous CBSCs from newborns with birth asphyxia. After parental consent, they treat patients with a combination of TH and transfusion of Sepax-isolated CBSCs at 24, 48 and 72 hours. We are currently conducting Japanese clinical trials of this combination therapy.

The original study protocol was developed and approved based on the Guidelines for the Use of Human Stem Cells in Clinical Trials. The umbilical cord is a rich source of blood (cord blood), and typically, a skilled person can obtain about 100 mL of blood. The blood is then processed in Sepax and aliquoted in three doses. Before actual transplantation the examination of those cells found that more than 90% of CD34⁺ stem cells were viable on day 3 (Nabetani et al., 2018).

Autologous CBSCs therapy for HIE: Autologous CBSCs therapy for neonatal HIE in addition to TH was performed in 6 newborn patients who were ≥ 36 weeks of gestational age and birth weight ≥ 1800 g with HIE and met the cooling criteria in Japan. All of them were discharged from the NICU without the support of a ventilator and survived from 2 to 5 years (Tsuji et al., 2020). Autologous CBSCs treatment has been shown to be feasible and safe in US (Cotten et al., 2014) and in Japan (Tsuji et al., 2020). In preterm infants autologous CBSCs were performed for not HIE but to prevent the development of prematurity-related complications, and obtained the same results, although this is a preliminary speculation based on small groups of very premature neonates who were 15 patients < 37 weeks of gestational age and 20 infants before 32 weeks of gestational age (Boruczowski et al., 2019).

Lee et al. (2012) reported that autologous CBSCs was administered to 20 patients with CP aged 2–10 years and 14 out of 20 patients

showed improvement. In 2015, Duke University reported that 63 patients with CP aged 2 years who had been administered autologous CBSCs intravenously and after one year showed no significant differences in the motor assessment was found between the study group and the control group, although there was a significant improvement in motor skills in the higher dose group than in the lower dose group.

Future perspectives: Survival with all three Bayley domain scores of ≥ 85 increased from 41% to 72% by combining cooling and autologous CBSCs transplantation (Cotten et al., 2014). This means that among every 1000 babies born with asphyxia, 400 will develop normally after hypothermia therapy alone, and 700 will develop normally when cell therapy is combined. This is a remarkable change. It also means that, however, hypothermia can save 400 babies but 600 will still likely develop CP. Cell therapy has the potential to prevent CP in 300 of these 600 patients. Still, the remaining 300 may develop CP. It seems impossible to decrease the number to zero (Figure 1A).

This is why we are seeking an additional cell-based approach particularly mesenchymal stem cells (MSCs), which can be isolated from the umbilical cord and cultured, and just a 1-cm piece of the cord is adequate (Mukai et al., 2019). Unlike cord blood, we can obtain enough cells even from premature babies. However autologous MSCs cannot be used to treat the acute phase of the first few days of life, as MSCs culture and quality control require a certain period of time. If treatment with MSCs is to be performed in the acute phase, the allogenic MSCs prepared in advance will be used instead of autologous MSCs, so it is necessary to pay sufficient attention to confirm the safety. While, because TH is only approved for mature infants born after 36 weeks of gestation, MSCs have potential for these preterm babies. If the efficacy of cell therapy is proven in combination with cooling, cell therapy alone may be beneficial as well. For example, small babies born at 34

week's gestation cannot receive TH but are able to receive CBSCs transplantation, if an adequate amount of cord blood is collected. For smaller babies, it would be difficult to always obtain an adequate amount of cord blood, but they still have the option of receiving MSCs. In 2016, an amplification peptide for CBSCs was discovered by Sugiyama et al. (patented but not published) and the possibility of treating premature infants with CBSCs has also emerged (Figure 1B).

Use of cord blood for regenerative medicine and cell therapy in Japan: The Japanese Society for CP Prevention is working to promote this new therapy across Japan in cooperation with healthcare centers. This is a new approach to treat the cause and prevent the future development of CP. Researchers at Kochi University, in contrast, have started a clinical trial of cord blood transplantation in children who have already developed CP. They are using autologous cord blood in six patients who had been stored at a private bank. While, Xie et al. (2016) reported that 12 HIE patients were treated with umbilical cord derived MSCs transplantation and markedly improvement of neurological function, cognition ability, emotional reaction and extrapyramidal function was observed compared to 10 control babies.

In regenerative medicine for HIE, a strategy will become necessary that cells such as MSCs, which strongly suppress inflammatory response, are administered in the acute phase, and thereafter cells such as CBSCs, which have angiogenic action, are administered for tissue recovery. It is desired to develop a combination therapy that makes use of the characteristics of each stem cell.

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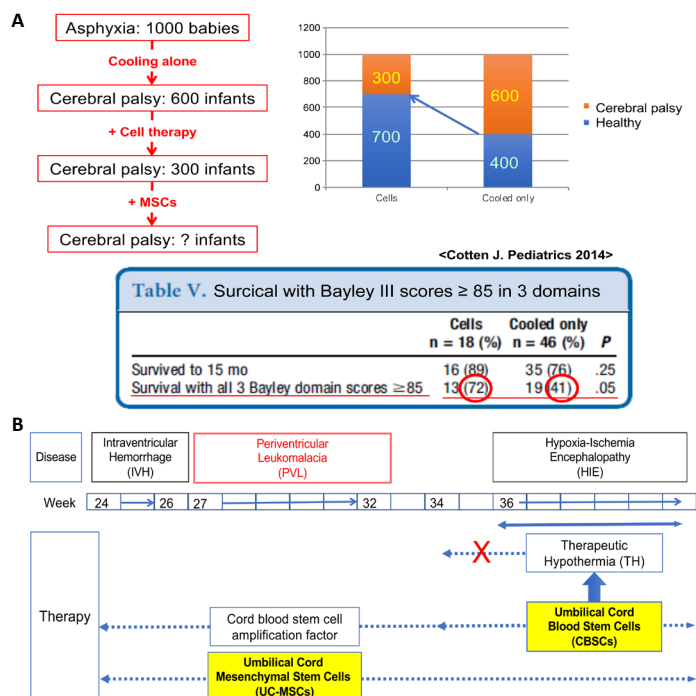


Figure 1 | Regenerative medicine provides new strategies for the treatment of perinatal brain injury. (A) Effects of cooling, cord blood stem cells and umbilical cord derived mesenchymal stem cells. (B) Perinatal therapeutic strategies for the prevention of cerebral palsy.

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