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ADVANCING CRITICAL CARE MEDICINE WITH STEM CELL THERAPY AND HYPOTHERMIA FOR CEREBRAL PALSY

Travis Dailey, **Yusef Mosley**, **Mibel Pabon**, **Sandra Acosta**, **Naoki Tajiri**, **Harry van Loveren**, **Yuji Kaneko**, and **Cesar V. Borlongan**
Department of Neurosurgery and Brain Repair, University of South Florida, College of Medicine, Tampa, Florida 33612 USA

Abstract

With limited clinical trials in stem cell therapy for adult stroke underway, the assessment of efficacy also needs to be considered for neonatal hypoxic-ischemic brain injury, considering its distinct symptoms. The critical nature of this condition establishes deficits that last a lifetime. Here, we will highlight the progress of current translational research, commenting on the critical nature of the disease, stem cell sources, the use of hypothermia, safety and efficacy of each treatment, modes of action, and the possibility of combination therapy. With this in mind, we reference translational guidelines established by a consortium of research partners called Stem cell Therapeutics as an Emerging Paradigm for Stroke (STEPS). The guidelines of STEPS are directed for evaluating outcomes for cell therapy in adult stroke; however, we identify the overlapping pathology, as we believe these guidelines will serve well in the investigation of neonatal hypoxic-ischemic therapy. Lastly, discussion into current treatment and a case report demonstrate that the capabilities for these treatments have arrived and the time for advancing stem cell therapy and hypothermia for cerebral palsy is now.

Keywords

cell transplantation; neonatal hypoxic-ischemic injury; translational research; clinical applications

Cerebral Palsy: The Critical Nature of the Disease

Given cerebral palsy (CP) is the most common motor disorder in children, with an estimated incidence of 1.5 to more than 4 per 1,000 live births worldwide [1], urgent research into translational therapy is essential in preventing the long-term cognitive and motor disabilities. These deficits are subsequent to conditions that may alter normal development or directly damage the developing brain of children. Historically, the etiology of the disease was heavily regarded as an interruption in oxygen delivery, producing a hypoxic-ischemic environment depriving neurological development of crucial oxygen. More recently, however, this paradigm began shifting to encompass more diverse risk factors that may lead to CP. These factors are further divided into congenital and acquired. Several currently considered risk factors for congenital CP subscribe to a gestational or peri-partum event inciting injury to the developing brain. Some of these factors include premature birth, low birth weight, multiple births, assisted reproduction, infections, jaundice, kernicterus, birth complications, and additional medical conditions of the mother. Many of these conditions

*Correspondence should be addressed to: Cesar V. Borlongan, Ph.D., Department of Neurosurgery, University of South Florida, Tampa, FL USA; Tel: 813-974-3154; Fax: 813-974-3078; cborlong@health.usf.edu.

Conflict of Interest: CVB has patents and pending patents on cell therapy.

may include fetal hypoxia such as: eclampsia, umbilical cord disruption, and even uterine rupture. Contrasting many of the risk factors for congenital CP, acquired CP follows events occurring further post-partum. These causes seem to follow a more direct incident, yet still include risk factors producing hypoxia. Brain infections (m meningitis), direct traumatic brain injury, and cerebral vascular disease may all predispose the developing brain to CP.

In addition to CP, hypoxic ischemic encephalopathy (HIE), neonatal encephalopathy (NE), and periventricular leukomalacia (PVL) all share a common denominator of neonatal hypoxic-ischemic brain injury. Often, children with hypoxic-ischemic brain injuries will present with neurodevelopmental deficits such as hearing and visual impairments, learning disabilities, and mental retardation. More specifically, early signs of CP include failure to reach motor or movement milestones and a stiff, floppy appearance in younger children. However, due to many of these signs being nonspecific, early developmental monitoring and screening is critical in obtaining a diagnosis. Additional observations in HIE include seizure onset beyond the first 12 hours of life , also associated with severe brain injury [2], further encouraging the necessity for prompt treatment.

Beyond just the morbidity, the mortality rates in hypoxic ischemic encephalopathy are staggering. Mortality rates are as high as 50% in HIE [3], with 25% of survivors displaying CP [4]. Furthermore, 30% of CP has been attributed to ischemic perinatal stroke [5].

The remainder of this review will outline the development of promising translational therapy options that seek to reduce the sequelae of CP (hereafter also referred to as HIE or neonatal hypoxic-ischemic injury), starting with cell-based therapies and then describing hypothermic treatments. We will conclude this review with a discussion of prospective future therapies, detailing a current case report involving the first autologous cell therapy for CP in a young boy caused by hypoxic-ischemic brain injury following cardiac arrest.

Cell Therapy as a Treatment for Neonatal Hypoxic-Ischemic Injury

Due to the homology that exists between the pathophysiology of neonatal hypoxic-ischemic brain injury and adult stroke, currently evolving cell-based therapies for stroke may prove to be effective in the younger populations. One such target for intervention includes exploration of the NMDA receptor function. Increased excitation coupled with aberrant oxidative stress, secondary to mitochondrial dysfunction, leads to the depletion of cellular energy of neurons in the brain of babies with HIE [6]; a condition referred to as “secondary energy failure” or “excitotoxic-oxidative cascade” [6]. Presently, the treatment protocol for HIE utilizes a reduction in metabolic demands, complementing the reduction in available energy, through the induction of hypothermia [7,8]. This treatment is most effective in newborns with a gestational age greater than or equal to 36 weeks [8], yet nearly half of all treated patients still have persistent deficits after therapy [8].

Transplantable Stem Cell Characteristics

In considering stem cell transplantation as a therapeutic option for HIE, the diverse characteristics of stem cells introduce a variety of treatment approaches. First, we must classify a well-defined phenotype to better understand the biology, mechanism of action, and evaluate safety and efficacy of a cell population [9]. Next, we need to consider how the theory of cell-based transplant therapy is evolving with emerging research. Originally, the concept of cell transplant in brain injury was thought to be a mechanism of neuronal cell replacement. This prospective is progressing to a more multi-faceted restorative mechanism, where transplanted cells may not only directly replace damaged cells but facilitate an environment conducive to endogenous repair through trophic, neurogenic, vasculogenic, angiogenic, and synaptogenic properties [10]. Cellular replacement and the aforementioned

bystander effects constitute the two major modes of action implicated in stem cell-mediated functional recovery in ischemic brain injury [11]. Lastly, we must consider the factors contributing to the development of a clinical product for treatment in a clinical setting, factors such as transplantation method and stem cell derivation. The ideal would be the availability of an injectable, autologous transplantation. Potential sites for autologous harvesting include craniofacial neural crest cells or from fibroblasts for generating induced pluripotent stem cells. The use of autologous cells would circumvent the potential for graft rejection. Prior preliminary studies have demonstrated safety in intravenous injection of autologous cord blood in children [12]. Additional potential sources for stem cell has been previously reviewed by our team [13].

Translational Study Protocols in Cell-Based Therapy for Neonatal Hypoxic-Ischemic Injury

Many variables contribute to designing translational studies, some of which include: harvesting stem cells, delivery method, optimal dose, and timing of transplantation. To best approximate a clinical setting, the laboratory parameters for testing should resemble clinical expectations to provide the best conditions for a translational approach. Conditions for harvesting stem cells include factors such as: autologous vs. allogenic, embryonic vs. extraembryonic, pluripotency vs. differentiated, and the potential for tumorigenesis. Another particular limitation is calculating a therapeutic cell dose while avoiding a potential microembolic event, seen at high dose injections. Thus, thorough testing and consideration must be obtained to balance the benefits vs. consequences. Furthermore, candidates for stem cell therapeutic products must employ a safe and efficacious delivery system. Minimally invasive delivery will prevent additional trauma to an already injured brain. As for timing of cell delivery, research suggests a neuroprotective phase of less than 1 day of injury and a neurorestorative phase of greater than 1 day after injury [14].

In an attempt to enhance the successful outcome of cell therapy in stroke patients, a consortium of academicians, industry partners and regulators, including the National Institutes of Health (NIH) and the U.S. Food and Drug Administration (FDA) have collaborated to produce the Stem cell Therapeutics as an Emerging Paradigm for Stroke (STEPS) [11,15–19]. Furthermore, the establishment of Baby STEPS strives to apply the same goals in cell therapy for neonatal hypoxic-ischemic brain injury [20].

Safety and Efficacy of Cell Therapy

When embarking on the search for novel therapeutics, safety and efficacy is of no exception. Cell-based therapy is of no exception. Some methods of assessing the safety and efficacy of experimental treatments in HIE include behavioral tests and histological assessment. Behavioral tests serve as an evaluation of motor and cognitive improvements for experimental groups while histological assays assess decreased cell loss/apoptosis, reduction of inflammation, neurogenesis, and suppressed oxidative stress; all of which may indicate modes of action and brain remodeling [21]. Further histological evaluation is necessary to analyze the characteristics, as discussed above, of the grafted cells within the hypoxic-ischemic tissue along with tumorigenesis or ectopic tissue formation [22].

The described assessments are needed both short- and long-term to examine the immediate and prolonged effects [23]. Such evaluations are complicated in neonatal HIE due to endogenous spontaneous recovery in both developmental and maturation periods in the neonatal animal [24] and pediatric patients [25].

Two clinical trials exist in the US (Duke University and Medical College of Georgia) to evaluate the safety and efficacy of umbilical cord blood transplants in CP patients. The transplantation of autologous cord blood in CP children has been established as safe; however, long-term follow-up is necessary to ensure there are no future complications [12,26].

Although cell-based therapy serves as a strong therapeutic foundation in the treatment of neonatal hypoxic-ischemic brain injury, there is much room for improvement. One such aspect for therapeutic exploration is the possibility of combined therapy, employing the metabolic benefits of hypothermia, detailed in the following sections, with the potential neuroprotective and neurorestorative benefits of a stem cell-based approach mentioned above.

Hypothermia as a Treatment for Neonatal Hypoxic-Ischemic Injury

In animal models of hypoxic-ischemic encephalopathy, hypothermia decreases glutamate release, attenuates secondary energy failure [7,27,28], normalizes protein synthesis, and reduces injury secondary to free radical production [29]. Hypothermia has further demonstrated neuroprotective effects against HIE events subsequent to aberrant stages of region-specific brain maturation [30], blood brain barrier (BBB) dysfunction, and mitochondrial impairment-induced apoptosis [31]. The exact mechanism of action underlying hypothermia is still unclear, but may include reduction in oxidative stress, energy deficits, and inflammation [32].

Hypothermic Treatment Characteristics

As discussed above, the observation of early seizure activity may indicate HIE, and possibly severe brain injury in newborns, prompting the notion of a critical relationship between onset of the symptoms and the start of therapy. Thus, any treatment modality, including hypothermia, is likely to exert its maximal benefit if induced rapidly, ideally within 6 hours after the hypoxic-ischemic injury and continuing over the following 12 hours or further [33].

Safety and Efficacy of Hypothermia

To date, small trials have yielded promising results in evaluating the safety of hypothermia in neonates [27]. Some of these randomized trials further demonstrated neurodevelopmental improvement in mild to moderate HIE. However, no significant improvement was seen in severe conditions of HIE in the neonatal population [7,8,34]. More recently, studies in the reduction of neurodevelopmental disability suggests a neuroprotective effect of hypothermia at 18 months of age with moderate or severe HIE [35].

Hypothermic neuroprotective approaches may demonstrate a reduction in the risk of developing neurodevelopmental disabilities, but areas of the brain already subjected to damage may still require a cell-based therapy, suggesting a tandem combination approach of both hypothermia and cell transplantation.

Combination Therapy of Cell Transplantation and Hypothermia

Currently, therapeutic options in human neonates suffering HIE seek to interrupt the cascade of events leading to injury, options including magnesium, calcium channel blockers, and NMDA receptor antagonists. These routes have yet to yield significant results in preventing neuronal loss [29,36]. As discussed above, in considering the established research of hypothermic and cell-based therapeutic options individually, combination therapy may yield greater functional recovery. As additional interventions emerge, such as erythropoietin and

helium [37,38], we may find that a composite mixture of treatment modalities could further enhance clinical efficacy as compared to stand-alone therapy.

Bone marrow-derived stem cells, such as mesenchymal stem cells (MSCs), have demonstrated mobilization, experimentally, into the periphery in response to physiological stress. Once in the periphery, these cells are recruited to areas of injury, as is the case of HIE [39]. Clinically, a benefit of mesenchymal stem cells is their potential ease for harvesting. Because they are present in umbilical cord blood, adipose tissue, amniotic fluid/tissue, and menstrual blood [40] there are multiple sources available for obtaining these cells. Furthermore, because of their immature immune status, the use of allogenic transplant (harvested from non-self) may prove to be as safe as an autologous transplant in terms of graft rejection. Prior research has demonstrated therapeutic effects of MSC transplantation against brain injury [41], although further research is essential in establishing their efficacy in the treatment of HIE and use for combination therapy.

Delta opioid agonists may resemble physiological states of hibernation, such as hypothermia [42], through direct opioid receptor and non-opioid mechanisms [43]. Substantiating a functional role of opioids, delta opioids may regulate neural stem and progenitor cell proliferation and differentiation [44], and possibly enhance cell-based therapeutics [45]. We recently demonstrated that moderate hypothermic treatment in an *in vitro* model of hypoxic-ischemic injury was enhanced by MSC treatment [46]. The combination therapy of moderate hypothermia and MSCs proved to be the optimal condition for preserving cell survival and mitochondrial activity after oxygen-glucose deprivation (OGD) conditions. Follow-up investigations into signaling pathways demonstrated growth factor upregulation and anti-apoptotic function complementing the observed benefits.

In spite of all the treatment options surfacing, the best predictor for favorable outcomes will likely be early detection of at-risk newborns. The ability to intervene prior to the pervasive damage caused by hypoxic-ischemic events may best facilitate the prevention of lifelong disabilities [47]

A First Case Report

Although stem cell-based therapy for neonatal hypoxic-ischemic brain injury is largely experimental, limited trials involving autologous umbilical cord blood cells for children with CP are underway. A case report recently published describes the use of human cord blood in neuroregeneration in a child with CP after suffering cardiac arrest. At two and half years of age the child suffered from global ischemic brain damage, secondary to cardiac arrest, resulting in a vegetative state. After an autologous intravenous transplant of the cord blood and rehabilitation, the child demonstrated exceptional motor recovery and a reduction in other symptoms at his two-month follow-up [48]. Researchers attribute this functional neuroregeneration to more than strictly rehabilitation, suggesting the potential for cell-based therapies has arrived.

Conclusion

Despite the promising results indicated by the case report above, extensive preclinical safety and efficacy research is still needed in extending these therapies to other neonatal diseases. This research begins with establishing standardized experiment models with quantitative assessments to predict clinical therapeutic capacity. The goals for the emergent future will be optimizing the aforementioned conditions. The optimal dose, delivery, and timing are under still under investigation. Current anecdotal reports of clinical improvement following cell therapy in children with CP or HIE should not compromise the Baby STEPS' footing on

the need for solid preclinical studies to support the clinical trials. The discussion above on the Baby STEPS guidelines may be applicable to other experimental therapies for neonatal hypoxic-ischemic injury [49] and should be used in concert with existing pediatric stroke recommendations for research and treatment interventions [50].

Experimental research for translational cell-based therapy continues to emerge for adult stroke. Although many of these principles may be applied to the neonatal population, the differences in pathophysiology warrant distinct trials to advance cellular therapy in this arena. Above, we have outlined the specific benefits of both stem cell therapy and hypothermia as innovative research modalities with direct clinical applications for CP. As with many current disease clinical treatment protocols, the use of a sole-stand-alone therapy is rare, necessitating the need for combination therapy. However, research on combination therapy remains an underexplored theme. Thus, future research is essential for determining any beneficial or harmful outcomes of combined therapy. In moving forward, these outcomes must be assessed for both behavioral and histological parameters. There is urgent demand to introduce novel therapies for children with CP. Given the critical and debilitating nature of the disease, we must remain proactive in advancing therapy from the bench to the bedside.

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