Review Article

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Cell-based treatment for perinatal hypoxic-ischemic encephalopathy

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Abstract:

Hypoxic-ischemic encephalopathy (HIE) is a major cause of acute neonatal brain injury and can lead to disabling long-term neurological complications. Treatment for HIE is limited to supportive care and hypothermia within 6 h injury which is reserved for full-term infants. Preclinical studies suggest the potential for cell-based therapies as effective treatments for HIE. Some clinical trials using umbilical cord blood cells, placenta-derived stem cells, mesenchymal stem cells (MSCs), and others have yielded promising results though more studies are needed to optimize protocols and multi-center trials are needed to prove safety and efficacy. To date, the therapeutic effects of most cell-based therapies are hypothesized to stem from the bystander effect of donor cells. Transplantation of stem cells attenuate the aberrant inflammation cascade following HIE and provide a more ideal environment for endogenous neurogenesis and repair. Recently, a subset of MSCs, the multilineage-differentiating stress-enduring (Muse) cells have shown to treat HIE and other models of neurologic diseases by replacing dead or ischemic cells and have reached clinical trials. In this review, we examine the different cell sources used in clinical trials and evaluate the underlying mechanism behind their therapeutic effects. Three databases–PubMed, Web of Science, and ClinicalTrials.gov–were used to review preclinical and clinical experimental treatments for HIE.

Keywords:

Cell therapy, cerebral palsy, hypoxic-ischemic encephalopathy, inflammation, regenerative medicine, stem cells

Introduction: Hypoxic-Ischemic Encephalopathy

Hypoxic-ischemic encephalopathy (HIE) is a subtype of neonatal encephalopathy caused by a perinatal asphyxial event and a major cause of acute neonatal brain injury with an incidence estimated at 1.5 per 1,000 live births.[1] Approximately 15%–25% of newborns affected by HIE die and 25% develop long-term neurological complications including behavioral, cognitive, and social deficits, cerebral palsy, and epilepsy.[2,3] Causes include uterine rupture, abruptio placenta, cord prolapse, placenta previa, maternal hypotension, shoulder dystonia, or breech presentation.[4] Symptoms of HIE include seizures, abnormal fetal heart rate, abnormal umbilical cord gases, need for immediate respiratory support, or low Apgar scores.[5]

Hypoxic-Ischemic Encephalopathy-Induced Brain Injury

The mechanism of brain injury following HIE involves an inflammatory response similar to adult ischemic stroke. However, HIE displays a unique immune cascade that stems from an immature neonatal immune and central nervous system.[6] HIE-induced brain injury occurs in two phases [Figure 1]. Primary phase of neuronal death is caused directly by the hypoxic-ischemic event and resulting metabolic failure. Exhaustion of adenosine triphosphate leads to the disruption of ion gradients and release of excitotoxic metabolites like glutamate.

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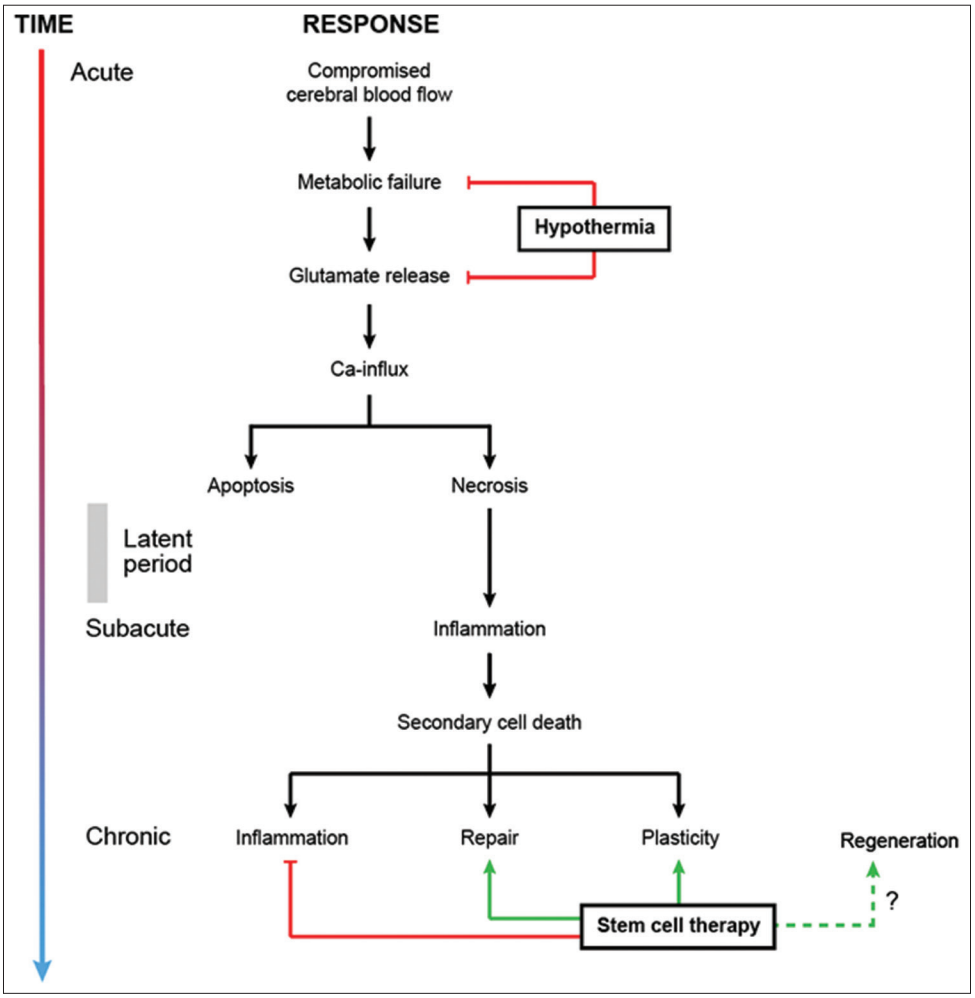

Figure 1: Inflammatory cascade following hypoxia-ischemia. Compromised cerebral blood flow leads to primary and secondary cellular death. The current treatment is limited to hypothermia which has a narrow therapeutic window. Stem cell therapy may provide a treatment option with a longer therapeutic window that targets multiple steps in the disease process

This is followed by a transient recovery of oxidative metabolism after reperfusion in the latent phase which typically lasts 6–15 h.[7] The latent period can be shorter depending on the severity of the primary injury and is considered to be the optimal window for therapeutic interventions. Cell death and injury triggers the inflammatory cascade which involves resident immune cells such as microglia and cerebral infiltration of peripheral immune cells, initiating the secondary phase of neuronal death through inflammatory cytokines and free radical production.[8] Chronic neuroinflammation in response to HIE may last for years in severe cases.[6]

Management and Treatment of Hypoxic-Ischemic Encephalopathy

The management of HIE in the acute setting aims to reduce primary brain injury and involves prompt resuscitation and supportive care including the prevention of hyperthermia, hypoglycemia, and seizures.[9] Although there is no cure for HIE, one treatment option available is hypothermia within 6 h of asphyxia event. Systemic or isolated head hypothermia (33°C–34°C) has been shown to reduce 12–22-month neurodevelopmental disability in randomized controlled clinical trials for full term infants.[10-12] The benefits are hypothesized to stem from the effect of hypothermia on multiple pathways involved in brain injury including excitatory amino acids, cerebral blood flow and metabolism, production of nitric oxides, and apoptosis.[12,13]

Despite hypothermia being implemented as the standard of care treatment for HIE, its narrow therapeutic window, lack of universal access, and limited effect in severe cases make the development of novel treatment for perinatal HIE an urgent clinical need.

Cell-Based Therapy

Here, we review the different cell sources used in experimental treatments for HIE as well as discuss the hypothesized mechanisms underlying their therapeutic

effects. Three databases–PubMed, Web of Science, and ClinicalTrials.gov–were used to find preclinical and clinical studies of cell-based treatments of HIE and other similar neurologic diseases.

Preclinical Studies

Of the experimental treatments currently being explored, cell-based therapy has gained much attention because of its relatively long therapeutic window and potential to target multiple facets of the disease such as promoting cell survival, anti-inflammation, repair, and regeneration [Figure 1]. Cell therapy using various types of stem cells including neural stem cells,[14,15] umbilical cord blood cells [Table 1],[16,17] and mesenchymal stem cells (MSCs)[18] have shown promising preclinical results. However, the suggested mechanism underlying the therapeutic effects of these cells is limited to the secretion of neurotrophic and anti-inflammatory cytokines which attenuate secondary cell death and promote recovery. Furthermore, transplanted cells have shown to only transiently engraft into host tissue.[19] Therefore, the search continues for a cell line that regenerates injured tissue and/or provides long-term therapeutic effects.

One emerging source for cell therapy is the multilineage-differentiating stress-enduring (Muse) cells. First described in 2010, Muse cells are a distinct subset of MSCs corresponding to several percent of total MSCs, and are found sporadically *in vivo* such as in the bone marrow, constituting ~ 0.03% of the mononucleated cell fraction, as well as in the peripheral blood and connective tissue of nearly every organ.[20,21] Muse cells are also found in extraembryonic tissue like the umbilical cord which make the distribution of this cell line distinct from other somatic stem cells.[21,22] Due to their nontumorigenicity, stress tolerance, and pluripotency, Muse cells have been explored for cell therapy in a broad range of diseases including acute myocardial infarcts, stroke, chronic kidney disease, and liver disease[23-26] and have reached clinical trials for stroke, acute myocardial infarction, epidermolysis bullosa, and spinal cord injury (Japan Pharmaceutical Information Center-Clinical Trials Information; JapicCTI-183834, 184103, 184563, and 194841).[27]

Recently, Suzuki *et al*. demonstrated the therapeutic effects of human Muse cells in a rat HIE model.[28] Seven-day-old rats underwent ligation of the left carotid artery and exposed to 8% oxygen for 1 h. At 72 h postligation, the animals received human Muse cells or non-Muse bone marrow MSCs through the right external jugular vein. Brain imaging analyses using ^{1}H-magnentic resonance spectroscopy showed that animals transplanted with Muse cells had a reduction in the release of excitotoxic metabolites such as glutamate and lactate when compared to the vehicle group. Furthermore, animals transplanted with Muse cells saw a reduction in microglial activation suggesting a dampened inflammatory response in the acute phase of HIE.

Although improvements following cell-based treatment for HIE models have been measured in previous studies, they failed to show long-term engraftment and regeneration of dead or damaged brain tissue following transplantation of donor cells.[14,16,17] Interestingly, Muse cells, but not non-Muse cell MSCs, showed long-term engraftment and expression of neural marker-positive cells (NeuN, MAP-2, and GST pi) until 6 months' posttransplantation without immunosuppression. Furthermore, these improvements were reflected in neurological function as assessed by locomotor activity and cognitive and motor functions in the animals. These results suggest the feasibility of intravenously administered donor-derived allogenic Muse cells for the treatment of HIE via cell replacement.

A previous clinical trial using IV autologous bone marrow mononuclear cells in children suffering from severe traumatic brain injury also showed benefits of stem cell therapy.[29] The treatment was associated with lower treatment intensity required to manage intracranial pressure, severity of organ injury, and duration of neurointensive care following injury (treatment n = 10, age and severity-matched controls = 19). Although the pathophysiology of severe traumatic brain injury and HIE are different, both disease processes involve secondary cell death due to inflammation which is the hypothesized therapeutic target based on preclinical studies.

Table 1: Current clinical trials assessing cell-based treatments for hypoxic-ischemic encephalopathy

Clinical trials government ID	Cell source	Sample size	Phase	Status on December 2020
NCT02455830	Autologous umbilical cord blood	18	NS	Active, not recruiting
NCT04063215	Adipose-derived mesenchymal stem cells	24	I, II	Recruiting
NCT04261335	Muse cells	12	I	Recruiting
NCT03352310	Autologous umbilical cord blood	40	I	Recruiting
NCT02551003	Autologous umbilical cord blood	60	I, II	Recruiting
NCT03635450	Umbilical cord tissue-derived mesenchymal stem cells	6	I	Active, not recruiting
NCT02434965	Autologous umbilical cord blood + human placenta-derived stem cells	20	II	Not yet recruiting

NS: Not significant

In 2013, Wang *et al*. reported the results of a clinical trial using bone marrow-derived MSCs in children with cerebral palsy, a common consequence of HIE.[30] In this study of 52 patients, the scores of gross motor function significantly increased at 1 month, 6 months, and 18 months after transplantation compared with baseline scores. As in the case the other cell sources mentioned, the hypothesized mechanism underlying the treatment's benefits is the secretion of trophic factors and cytokines that attenuates inflammation and promotes neurogenesis.

While the search for stem cell-based therapy for HIE is still at its nascent stage, significant strides have been made owing to the progress made in regenerative medicine for neurologic diseases as a whole. Two hypothesized modes of action involved in stem cell-mediated functional recovery in HIE are the by-stander effect and cell replacement. So far, there has not been a donor cell line that has been proven to regenerate dead or ischemic brain tissue in the setting of HIE. Based on preclinical data, Muse cells may fill this niche which would be a huge step in progress for the field of regenerative medicine. As in the case with preclinical studies using HIE models, IV administration in rodent lacunar stroke models have shown donor cell migration, differentiation, and engraftment to the peri-infarct area as well as long-term functional recovery.[31] Clinical trials using IV donor-derived Muse cells (called CL2020 in the clinic) are ongoing for the treatment of myocardial infarction, stroke, and epidermolysis bullosa. Because human Muse cells express human leukocyte antigen (HLA)-G, an inhibitory receptor relevant to immunomodulation in the placenta, allogenic Muse cells may survive in the host tissue long-term without immunosuppression.[32] These small clinical trials advance the use of allogenic stem cells without HLA-matching or long-term immunosuppression – an option that may be more practical than an autologous approach since the latter requires more time and resources.

Conclusion

Cell-based therapies sourced from autologous umbilical cord blood, placenta-derived stem cells, and MSCs are leading to new protocols for the treatment of HIE. To date, the main mechanism of action of these experimental treatments is the secretion of neurotropic and anti-inflammatory cytokines and molecules that promote neurogenesis and repair. Muse cells, a subset of MSCs, are endogenous reparative stem cells reported in preclinical studies of multiple diseases as capable of differentiating and engrafting into host tissue long-term. Currently, clinical trials using IV administered Muse cells is ongoing for the treatment of HIE and stroke. Results from these studies may shed light on the feasibility of cell replacement using stem cells to treat neurologic diseases.

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Conflicts of interest

Prof. Cesario V. Borlongan is Associate Editor of *Brain Circulation*.

References

1. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev 2010;86:329-38.
2. Volpe JJ. Neonatal encephalopathy: An inadequate term for hypoxic-ischemic encephalopathy. Ann Neurol 2012;72:156-66.
3. Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. J Biomed Biotechnol 2011;2011:609813.
4. Allemand F, Reale F, Sposato M, Allemand A. Perinatal hypoxic-ischemic encephalopathy: Epileptic and paretic outcome at one year of age. Ital J Pediatr 2009;35:14.
5. Allen KA, Brandon DH. Hypoxic ischemic encephalopathy: Pathophysiology and experimental treatments. Newborn Infant Nurs Rev 2011;11:125-33.
6. Liu F, McCullough LD. Inflammatory responses in hypoxic ischemic encephalopathy. Acta Pharmacol Sin 2013;34:1121-30.
7. Bennet L, Tan S, Van den Heuij L, Derrick M, Groenendaal F, van Bel F, *et al*. Cell therapy for neonatal hypoxia-ischemia and cerebral palsy. Ann Neurol 2012;71:589-600.
8. Li B, Concepcion K, Meng X, Zhang L. Brain-immune interactions in perinatal hypoxic-ischemic brain injury. Prog Neurobiol 2017;159:50-68.
9. Glass HC, Ferriero DM. Treatment of hypoxic-ischemic encephalopathy in newborns. Curr Treat Options Neurol 2007;9:414-23.
10. Eicher DJ, Wagner CL, Katikaneni LP, Hulsey TC, Bass WT, Kaufman DA, *et al*. Moderate hypothermia in neonatal encephalopathy: Efficacy outcomes. Pediatr Neurol 2005;32:11-7.
11. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, *et al*. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: Multicentre randomised trial. Lancet 2005;365:663-70.
12. Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, *et al*. Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. N Engl J Med 2005;353:1574-84.
13. Wisnowski JL, Wu TW, Reitman AJ, McLean C, Friedlich P, Vanderbilt D, *et al*. The effects of therapeutic hypothermia on cerebral metabolism in neonates with hypoxic-ischemic encephalopathy: An *in vivo* 1H-MR spectroscopy study. J Cereb Blood Flow Metab 2016;36:1075-86.
14. Sato Y, Nakanishi K, Hayakawa M, Kakizawa H, Saito A, Kuroda Y, *et al*. Reduction of brain injury in neonatal hypoxic-ischemic rats by intracerebroventricular injection of neural stem/progenitor cells together with chondroitinase ABC. Reprod Sci 2008;15:613-20.
15. Sato Y, Oohira A. Chondroitin sulfate, a major niche substance of neural stem cells, and cell transplantation therapy of neurodegeneration combined with niche modification. Curr Stem Cell Res Ther 2009;4:200-9.
16. Hattori T, Sato Y, Kondo T, Ichinohashi Y, Sugiyama Y, Yamamoto M, *et al*. Administration of umbilical cord blood cells

transiently decreased hypoxic-ischemic brain injury in neonatal rats. Dev Neurosci 2015;37:95-104.
17. Nakanishi K, Sato Y, Mizutani Y, Ito M, Hirakawa A, Higashi Y. Rat umbilical cord blood cells attenuate hypoxic-ischemic brain injury in neonatal rats. Sci Rep 2017;7:44111.
18. Sugiyama Y, Sato Y, Kitase Y, Suzuki T, Kondo T, Mikrogeorgiou A, *et al*. Intravenous administration of bone marrow-derived mesenchymal stem cell, but not adipose tissue-derived stem cell, ameliorated the neonatal hypoxic-ischemic brain injury by changing cerebral inflammatory state in rat. Front Neurol 2018;9:757.
19. Trounson A, McDonald C. Stem cell therapies in clinical trials: Progress and challenges. Cell Stem Cell 2015;17:11-22.
20. Kuroda Y, Kitada M, Wakao S, Nishikawa K, Tanimura Y, Makinoshima H, *et al*. Unique multipotent cells in adult human mesenchymal cell populations. Proc Natl Acad Sci U S A 2010;107:8639-43.
21. Wakao S, Kushida Y, Dezawa M. Basic characteristics of muse cells. Adv Exp Med Biol 2018;1103:13-41.
22. Leng Z, Sun D, Huang Z, Tadmori I, Chiang N, Kethidi N, *et al*. Quantitative analysis of ssea3+cells from human umbilical cord after magnetic sorting. Cell Transplant 2019;28:907-23.
23. Minatoguchi S, Mikami A, Tanaka T, Minatoguchi S, Yamada Y. Acute myocardial infarction, cardioprotection, and muse cells. Adv Exp Med Biol 2018;1103:153-66.
24. Niizuma K, Borlongan CV, Tominaga T. Application of muse cell therapy to stroke. Adv Exp Med Biol 2018;1103:167-86.
25. Uchida N, Kumagai N, Kondo Y. Application of muse cell therapy for kidney diseases. Adv Exp Med Biol 2018;1103:199-218.
26. Nishina T, Hoshikawa KT, Ueno Y. Current cell-based therapies in the chronic liver diseases. Adv Exp Med Biol 2018;1103:243-53.
27. Noda T, Nishigaki K, Minatoguchi S. Safety and efficacy of human muse cell-based product for acute myocardial infarction in a first-in-human trial. Circ J 2020;84:1189-92.
28. Suzuki T, Sato Y, Kushida Y, Tsuji M, Wakao S, Ueda K, *et al*. Intravenously delivered multilineage-differentiating stress enduring cells dampen excessive glutamate metabolism and microglial activation in experimental perinatal hypoxic ischemic encephalopathy. J Cereb Blood Flow Metab 2020:271678X20972656. Advance online publication.
29. Liao GP, Harting MT, Hetz RA, Walker PA, Shah SK, Corkins CJ, *et al*. Autologous bone marrow mononuclear cells reduce therapeutic intensity for severe traumatic brain injury in children. Pediatr Crit Care Med 2015;16:245-55.
30. Wang X, Cheng H, Hua R, Yang J, Dai G, Zhang Z, *et al*. Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: A preliminary clinical study. Cytotherapy 2013;15:1549-62.
31. Abe T, Aburakawa D, Niizuma K, Iwabuchi N, Kajitani T, Wakao S, *et al*. Intravenously transplanted human multilineage-differentiating stress-enduring cells afford brain repair in a mouse lacunar stroke model. Stroke 2020;51:601-11.
32. Wakao S, Akashi H, Kushida Y, Dezawa M. Muse cells, newly found non-tumorigenic pluripotent stem cells, reside in human mesenchymal tissues. Pathol Int 2014;64:1-9.