



Preventing Brain Damage from Hypoxic–Ischemic Encephalopathy in Neonates: Update on Mesenchymal Stromal Cells and Umbilical Cord Blood Cells

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Abstract

Keywords

- ▶ hypoxic–ischemic encephalopathy
- ▶ cerebral palsy
- ▶ umbilical cord blood stem cells
- ▶ mesenchymal stromal cells
- ▶ HIE
- ▶ CP
- ▶ MSCs
- ▶ secondary energy failure

Neonatal hypoxic–ischemic encephalopathy (HIE) causes permanent motor deficit “cerebral palsy (CP),” and may result in significant disability and death. Therapeutic hypothermia (TH) had been established as the first effective therapy for neonates with HIE; however, TH must be initiated within the first 6 hours after birth, and the number needed to treat is from 9 to 11 to prevent brain damage from HIE. Therefore, additional therapies for HIE are highly needed. In this review, we provide an introduction on the mechanisms of HIE cascade and how TH and cell therapies such as umbilical cord blood cells and mesenchymal stromal cells (MSCs), especially umbilical cord-derived MSCs (UC-MSCs), may protect the brain in newborns, and discuss recent progress in regenerative therapies using UC-MSCs for neurological disorders. The brain damage process “HIE cascade” was divided into six stages: (1) energy depletion, (2) impairment of microglia, (3) inflammation, (4) excitotoxicity, (5) oxidative stress, and (6) apoptosis in capillary, glia, synapse and/or neuron. The authors showed recent 13 clinical trials using UC-MSCs for neurological disorders. The authors suggest that the next step will include reaching a consensus on cell therapies for HIE and establishment of effective protocols for cell therapy for HIE.

Key Points

- This study includes new insights about cell therapy for neonatal HIE and CP in schema.
- This study shows precise mechanism of neonatal HIE cascade.
- The mechanism of cell therapy by comparing umbilical cord blood stem cell with MSC is shown.
- The review of recent clinical trials of UC-MSC is shown.

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Neonatal mortality rates have decreased considerably over the past several decades, yet the prevalence of severe neurological sequelae such as cerebral palsy (CP), epilepsy, intellectual disability, respiratory disorders, hearing loss, visual disturbances, hydrocephalus, behavioral problems and others due to hypoxic-ischemic encephalopathy (HIE) have remained at a similar rate over several decades. HIE in full-term infants occurs in an estimated 0.5 to 2/1,000 live births and results in severe disability and even death. In recent years, therapeutic hypothermia (TH) has been established as the first effective therapy for neonates with HIE.¹ However, TH must be initiated within the first 6 hours of birth, and the number needed to treatment is 9 to 11 to prevent brain damage from HIE. Therefore, additional therapies for HIE are highly needed. In this context, cell therapies such as umbilical cord blood stem cells (UCBCs), bone marrow (BM) stem cells, and umbilical cord/BM-derived mesenchymal stromal cells (UC/BM-MSCs) have started to be incorporated into new protocols for protecting against ischemic brain damage.² Interestingly, MSC therapy may also provide promising results for neonates with acute respiratory distress syndrome in the coronavirus disease 2019 infection era.³

The pathological characteristics of neonatal brain injury differ markedly from those in adults. For example, energy demand is much smaller hypoxia and brain swelling is not as precarious and there is a secondary energy failure phase due to complicated cascade of HIE.⁴⁻⁶ Furthermore, there is a need to overcome “secondary energy failure,” since newborns with HIE often deteriorate even after appropriate neonatal cardiopulmonary resuscitation (CPR), respiratory circulation support therapy, and TH.

Mechanism of Neonatal HIE Cascade

We present the mechanism of the HIE cascade, energy depletion, excitotoxicity, intracellular Ca^{2+} mobilization, and cell damage process in **Fig. 1**.

Olney first reported “excitotoxicity.” Meaning that some of the neural cell death due to hypoxia-ischemic (HI) insult was mediated by excess production of the excitatory neurotransmitter “glutamate” and elevation of intracellular Ca^{2+} concentration by N-methyl-D-aspartate glutamatergic receptor (NMDA GluR), AMPA/Kainate GluR (A/K GluR), and metabotropic GluR(mGluR) in different ways.^{7,8} We summarize the mechanism of “excitotoxicity” in **Fig. 1**. Glutamate (Glu) is converted to Glutamine (Gly) by the action of glutamine synthetase (GS) in astrocyte, and shuttled from astrocyte to neurons, then converted to Glu by glutaminases (GLS). Energy depletion in presynaptic site of neurons activates release of Glu into synapse. A large proportion of the Glu released at the synapse is taken up by astrocytes via excitatory amino acid transporter together with three Na^+ ions. This Na^+ is extruded by the action of the Na^+/K^+ ATPase. Glu uptake cannot work enough in the condition of adenosine triphosphate (ATP) reduction. Glu at synapse activates NMDA GluR, A/K GluR, and mGluR.

In contrast to the experience with adult HI insults, some newborns who had recovered from severe asphyxia subsequently deteriorated rapidly and expired a few days later. Kirino first reported the phenomenon “Delayed neuronal

death.”⁹ Delpy et al reported the phenomenon of delayed neuronal death in newborns after HI insults, termed “secondary energy failure,” using a phosphorus magnetic resonance spectroscopy to replicate the complicated process in piglets and rat pups in the 1990s.¹⁰⁻¹³ Simon et al proposed that brain damage due to HIE can be treated by NMDA antagonists and suggested that brain damage due to HIE could be blocked pharmacologically to protect against neonatal HIE.¹⁴ Unfortunately, NMDA receptor blocker and other drugs, such as calcium channel antagonists and magnesium sulfate, were not effective in clinical care. We reported that irreversible neuronal cell damage was triggered by an elevation of intracellular Ca^{2+} concentration subsequent to excessive accumulation of the excitatory neurotransmitter glutamate in immature and mature rats during ischemia and glucose deprivation.¹⁵ Furthermore, there is increasing evidence that mitochondrial dysfunction generated by excessive intracellular Ca^{2+} accumulation results in oxidative stress, apoptosis, and necrosis. We summarize the mechanism of elevation of intracellular Ca^{2+} concentration and irreversible neuronal cell death in **Fig. 1**. Calcium influx by NMDA GluR directly increases intracellular Ca^{2+} concentration. A/K receptors flux large amounts of sodium, depolarizing cell membrane, and blocking Ca^{2+} efflux from neurons by Cation/ Ca^{2+} antiporter. Depolarization of cell membrane activates voltage-sensitive Ca channels and facilitates NMDA GluR activation. Signaling of mGluR by Glu, activating phospholipase C (PLC), facilitates inositol 1,4,5-triphosphate (IP3) and activates IP3 inducing calcium release from endoplasmic reticulum (ER). Therefore, activation of three different GluRs leads to elevation of intracellular Ca^{2+} concentration in different ways. Furthermore, elevation of intracellular Ca^{2+} concentration activates calcium-induced calcium release from ER. Ca^{2+} efflux from neurons by Ca^{2+} -ATPase cannot work enough to prevent elevation of intracellular Ca^{2+} concentration in the condition of ATP deduction. These multiple mechanisms of HIE induce steep elevation of intracellular Ca^{2+} concentration from 10^{-7}M in the condition of ATP reduction and reach plateau level in several minutes as we show in our article. Elevation of intracellular Ca^{2+} concentration deteriorates mitochondrial function and leads to accumulations of free radicals, necrosis, and apoptosis. After plateau level of intracellular Ca^{2+} concentration, neuronal damage becomes irreversible. So, recent therapies in the acute phase of HIE could be expected with the aim of suppressing the commencement of brain damage cascade.

On the contrary, we reported that the presence of glucose is essential for neural activity in the adult rat and showed that glucose metabolites such as lactate and β -hydroxybutyrate (OHBA) are available for both neural activity and maintaining the level of high-energy phosphates in a tissue of hippocampus slice in the immature rats.¹⁶ We also reported the possibility of lactate preserving neural function of the adult brain.¹⁷ We further described the relationship between neural activity and the levels of high energy phosphates during deprivation of oxygen and/or glucose in hippocampal slices of immature and adult rats. Our results indicate that the immature rat is extremely resistant to

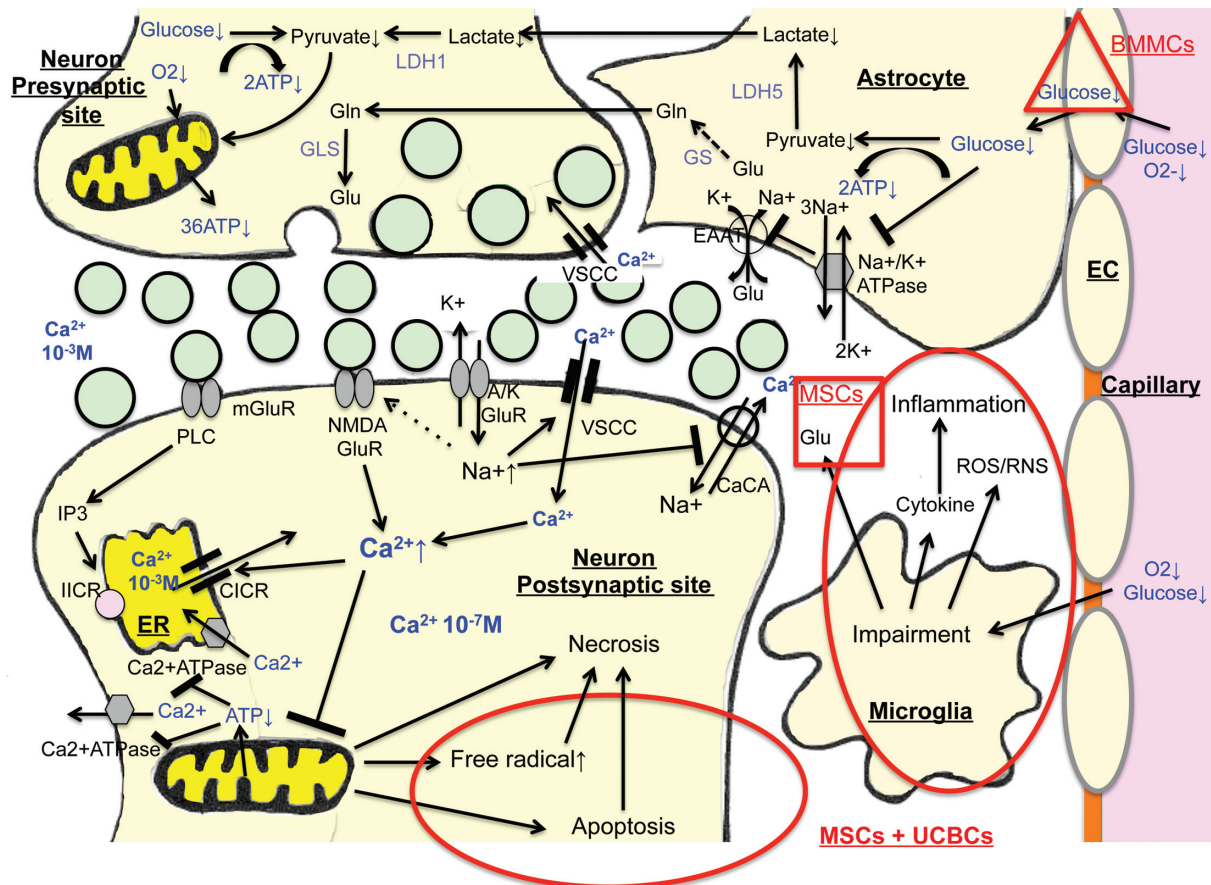


Fig. 1 Mechanism of hypoxic-ischemic encephalopathy (HIE) cascade and cell therapy for HIE cascade. First, HIE induces energy depletion (O₂-, Glucose-) in a capillary and glucose reduction in endothelial cell (EC) and in astrocyte. Glucose reduction in astrocyte leads to reduction of pyruvate and lactate that is converted from pyruvate by lactate dehydrogenase 5 (LDH5). And it leads to reduction of lactate and reduction of pyruvate that is converted from lactate-by-lactate dehydrogenase 1 (LDH1) in presynaptic site. Then, pyruvate reduction with O₂ reduction leads to adenosine triphosphate (ATP) reduction in mitochondria. Glutamate (Glu) is converted to glutamine (Gln) by the action of glutamine synthetase (GS) in astrocyte, and shuttled from astrocyte to neurons, then converted to Glu by glutaminases (GLS). Energy depletion in presynaptic site of neurons activates release of Glu into synapse. A large proportion of the Glu released at the synapse is taken up by astrocytes via excitatory amino acid transporter (EAAT) together with three Na⁺ ions. This Na⁺ is extruded by the action of the Na⁺/K⁺ ATPase. Glu uptake cannot work enough in the condition of ATP reduction.⁸³ Glu at synapse activate N-methyl-D-aspartate glutamate glutamatergic receptor (NMDA GluR), AMPA/Kainate glutamatergic receptor (A/K GluR), and metabolic glutamatergic receptor (mGluR). On the other hand, A/K receptors do not directly allow entry of sufficient calcium to increase intracellular Ca²⁺ concentration. However, A/K receptors flux large amounts of sodium, depolarizing cell membrane and blocking Ca²⁺ efflux from neurons by cation/ Ca²⁺ antiporter (CaCA). Depolarization of cell membrane activates voltage-sensitive Ca channels (VSCC) and facilitate NMDA GluR activation. Signaling of mGluR by Glu, activating phospholipase C (PLC), facilitate inositol 1,4,5-triphosphate (IP3) and activate IP3 induces calcium release (IICR) from endoplasmic reticulum (ER). Furthermore, elevation of intracellular Ca²⁺ concentration activates calcium-induced calcium release (CICR) from ER. Ca²⁺ efflux from neurons by Ca²⁺-ATPase cannot work enough to prevent elevation of intracellular Ca²⁺ concentration in the condition of ATP deduction. These multiple mechanisms of HIE induce steep elevation of intracellular Ca²⁺ concentration from 10⁻⁷M and reach plateau level in several minutes as we show in our article. After plateau level of intracellular Ca²⁺ concentration, neuronal damage becomes irreversible.¹⁵ Microglia also play an important role of neuronal cell damage in case of HIE. Energy depletion in a capillary induces impairment of microglia and it facilitates cytokines, Glu, reactive oxygen species (ROS), and reactive nitrogen species (RNS). Red circles show protective effects of mesenchymal stem cell (MSC) and umbilical cord blood stem cells (UCBCs) on impairment of microglia, inflammation, oxidative stress (free radicals and ROS/RNS) and apoptosis. Red square shows a protective effect of MSC on excitotoxicity. Red triangle shows a protective effect of bone marrow mononuclear cells (BM-MNCs) on energy reduction in EC via gap junction.⁵⁵

oxygen deprivation from a functional and metabolic perspectives, whereas in the adult rat, preservation of neural activity highly depends on both oxygen and glucose and that glucose plays an important role in the preservation of neural activity in addition to its major function as an energy substrate, especially in immature animals.⁶ Therapies targeting energy substrates are still the focus of current research.¹⁸

Ferriero explained that brain damage in the HIE cascade is divided into five stages: (1) energy depletion, (2) inflammation,

(3) excitotoxicity, (4) oxidative stress, and (5) apoptosis.¹⁹⁻²¹ Recent reports not only focus on microglia that have a role in immunomodulation but also promote local network synchronization in the synapses in the developing brain.²² Impairment of microglia plays a key role in most early stages of HIE cascade, especially in terms of inflammation and that may continue during a period of days to weeks. We summarize the mechanism of impairment of microglia in case of HIE in **-Fig. 1**. Energy depletion (O₂-, Glucose-) in a capillary induces impairment of

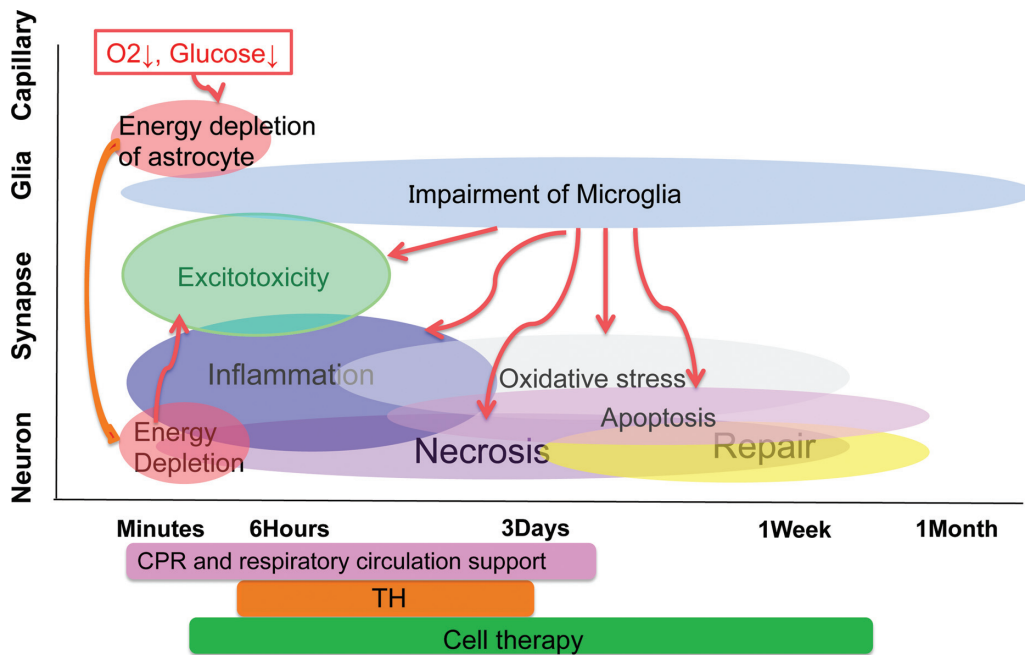


Fig. 2 Timing of promising cell therapies with standard therapies for hypoxic–ischemic encephalopathy cascade. Inflammation, oxidative stress, apoptosis, and necrosis occur through downstream energy depletion, excitotoxicity, and/or impairment of microglia. Cell damage begins immediately after hypoxic–ischemic insult and repair process begin after that. Impairment of microglia, oxidative stress, and apoptosis continues during a period of days to weeks beyond the phase of “secondary energy failure.” Cell therapy could also work for days to weeks after neonatal cardiopulmonary resuscitation (CPR), respiratory circulation support therapy, and therapeutic hypothermia (TH) are over.

microglia and it facilitates cytokines, Glu, reactive oxygen species (ROS), and reactive nitrogen species (RNS). Therefore, we suggest an additional mechanism namely “Impairment of microglia” and divide the brain damage process into six stages: (1) energy depletion, (2) impairment of microglia, (3) inflammation, (4) excitotoxicity, (5) oxidative stress, and (6) apoptosis (→ Fig. 2). Impairment of microglia, oxidative stress, and apoptosis might continue during a period of days or weeks after CPR and respiratory circulation support, so further strategies are needed for this period of time.

How TH and Cell Therapy Prevent Newborn Brain Damage from HIE?

In 1989, Busto et al showed that mild hypothermia after HI insult in adult rats reduced the release of neurotransmitters and had a protective effect on hippocampal neuronal injury.²³ In 1996, Thoresen et al reported the protective effect of hypothermia against brain injury in neonatal rats.^{24,25} In 1997, we demonstrated that hypothermia therapy was an

effective treatment for hypoxic or ischemic brain damage in rats by suppressing energy loss and elevation of intracellular Ca^{2+} concentration. The protective effects of hypothermia (33 and 29°C) on the neuronal activity, intracellular Ca^{2+} accumulation, and ATP levels during deprivation of oxygen and/or glucose were investigated using guinea pig hippocampal slices.²⁶ In 2004, McManus et al reported that neuroprotective effects of hypothermia are mediated through a reduction in nitric oxide and superoxide formation and that this effect is likely to be downstream of NMDA receptor activation.²⁷ Recent studies showed that TH has been suggested to provide a protective effect mainly on (1) energy depletion due to reduction in energy metabolism but also other five stages (2) impairment of microglia,²⁸ (3) inflammation,^{29,30} (4) excitotoxicity,²⁷ (5) oxidative stress,²⁷ and (6) apoptosis³¹ (→ Table 1).

In recent years, cell therapies such as UCBCs, umbilical cord-derived mesenchymal stromal cells (UC-MSCs) and bone marrow-derived mesenchymal stromal cells (BM-MSCs) are attracting attention due to their HIE neuroprotective ability.

Table 1 Stage of effectiveness of TH, UCBCs, and MSCs for HIE

	Energy depletion	Impairment of Microglia	Inflammation	Excitotoxicity	Oxidative stress	Apoptosis	Enhance regeneration
TH	++ ²⁶	+ ²⁸	+ ^{29,30}	+ ²⁷	+ ²⁷	+ ³¹	
UCBCs	+ ⁵⁹	+ ⁴²	+ ⁴⁸		+ ⁴⁹	+ ⁴⁹	+ ^{51,53,57}
MSCs		++ ^{33,43,44}	++ ^{39,44–47}	+ ³⁴	+ ³⁴	+ ³⁴	+ ³⁵

Abbreviations: HIE, hypoxic–ischemic encephalopathy; MSCs, mesenchymal stem cells; TH, therapeutic hypothermia; UCBCs, umbilical cord blood stem cells.

UCBCs are suggested to provide a protective effect mainly on impairment of microglia, inflammation, oxidative stress and apoptosis, as well as their ability to enhance regeneration. Red circles in **Fig. 1** show protective effects of UCBCs on impairment of microglia, inflammation, oxidative stress (free radicals and ROS/RNS), and apoptosis. While red triangle in **Fig. 1** shows a protective effect of bone marrow mononuclear cells (BM-MNCs) on energy reduction in endothelial cell via gap junction (55) (**Table 1**).

MSCs are cells derived from several sources as defined with by the International Society for Cellular Therapy; first, MSCs must be plastic-adherent when maintained in standard culture conditions. Second, MSC must express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79 α , or CD19 and human leukocyte antigen-DR isotype (HLA-DR) surface molecules. Finally, MSC must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro.³² MSCs may provide a protective effect against impairment of microglia,³³ inflammation, excitotoxicity, oxidative stress, and apoptosis³⁴ Red circles in **Fig. 1** show protective effects of MSC on impairment of microglia, inflammation, oxidative stress (free radicals and ROS/RNS), and apoptosis. And, red square in **Fig. 1** shows a protective effect of MSC on excitotoxicity (**Table 1**). Furthermore, the paracrine effect by neurotrophic factors has been reported in UC-MSCs on neurological regeneration, showing that UC-MSCs-conditioned medium enhances Schwann cell's viability and proliferation via increases in nerve growth factor and brain-derived neurotrophic factor (BDNF) expression.³⁵ We also found that UC-MSCs secrete BDNF and hepatocyte growth factor (HGF) attenuating brain injury.^{34,36} We also hope that MSC-derived extracellular vesicles will be therapeutic candidates for a successful clinical translation.³⁷

However, some reports propose negative effects of cell therapy. Dalous et al demonstrated that UC-MNCs cannot integrate into the developing brain or promote subsequent repair in most conditions tested, and that the intraperitoneal injection of high amounts of UC-MNCs aggravated WMD and was associated with systemic inflammation.³⁸ Furthermore, the timing, dose, route of application of UC-MNCs, and UC-MSCs have not yet been precisely determined. A systematic review and meta-analysis of MSC for HIE demonstrated that there is various delivery routes (intracerebral, intranasal, intravenous, and others), various timing of application after HI insult (≤ 72 hours, >72 hours, multiple doses), various source (BM, UC, placenta, not reported), various origin (Allogeneic, Xenogeneic), and various dose ($\leq 250,000$ cells, $>250,000$ cells– $\leq 500,000$ cells, $>500,000$ cells– $\leq 1,000,000$ cells, $>1,000,000$ cells).³⁹ Interestingly, combination of TH and other therapy might worsen the brain injury.⁴⁰ It is still highly controversial whether cell therapy for perinatal brain injury is effective or not.

Reduction in Impairment of Microglia

Microglia, immune cells of the central nervous system, continuously survey the microenvironment and respond to brain injury.⁴¹ Microglia are activated in response to brain injury, and are polarized toward an inflammatory phenotype that enhances the generation of pro-inflammatory media-

tors such as interleukin-1 β and tumor necrosis factor- α . However, they can also be polarized to the anti-inflammatory phenotype via mediators such as arginase 1 and transforming growth factor- β . Therefore, modulation of the phenotype of the microglia may be a novel therapeutic strategy for the treatment of neurological disorders accompanied by inflammation. Li et al reported that UCBC administration at 12 hours after HI reduces white matter injury by affecting activated microglia.⁴² Recent experimental studies reported that MSCs affect activated microglia.⁴³ We found UC-MSCs could immunomodulate activated microglia and decreased their inflammatory cytokines. Moreover, UC-MSCs could change their phenotypes including morphology and phagocytic ability. Morphological and phagocytotic analyses revealed that lipopolysaccharide stimulation significantly changed microglial morphology to amoeboid in which F-actin spread with ruffle formation resulting in reduced phagocytosis of *Escherichia coli*, while MSC co-culture induced shrinkage and concentration of F-actin to form an actin ring, thereby restoring phagocytosis.⁴⁴ These effects of UC-MSCs that modulate the activated microglia may be a therapeutic potential for the treatment of neurological disorders accompanied by inflammation.

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. Immunosuppressive effects have now become the most popular property of MSCs for clinical use.⁴⁵ Defect of HLA-DR (class II) expression in MSCs can theoretically rescue them from immune recognition by CD4+ T cells.⁴⁶ Moreover, MSCs do not express co-stimulatory surface antigens such as CD80, and CD86, which activate T-cells.⁴⁷ Thus, MSCs escape activated T cells and exert immunomodulation. MSCs are reported to protect brains against global and local neuroinflammatory cascades triggered by HI events.^{39,44} However, at times MSCs have both anti- and proinflammatory effects. Indeed, in our study, some UC-MSCs caused inflammatory response in resting-surveilling (not activated) microglia, indicating that MSCs should not be administered to healthy brain with no inflammation.⁴⁴ Some reports, however, suggest that UCBC administration reduces white matter injury after HI insult, via a combination of anti-inflammatory and other actions.⁴⁸

Reduction of Oxidative Stress and Apoptosis

Hattori et al reported that a single intraperitoneal injection of UCB-derived mononuclear cells 6 hours 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. They suggested that repeated administration or a combination treatment of UCB-derived mononuclear cells may be required to achieve sustained protection.⁴⁹ However, MSCs are reported to be able to alleviate oxidative stress, and to reduce apoptosis.

We also reported UC-MSC-secreted HGF and BDNF have neuroprotective effects on damaged neurons by reducing the number of neurons displaying signs of apoptosis/necrosis.³⁴

Enhancement of the Regenerative Process by Secretion of Various Cytokines

Human CD34+ cells have been shown to secrete various growth factors such as BDNF, glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and numerous angiogenic factors, including HGF and insulin-like growth factor-1.^{50–53} MSCs have also been reported to secrete various neurotrophic factors and growth factors such as BDNF, GDNF, HGF, and VEGF.³⁵

Enhancement of the Regenerative Process by Angiogenesis for Better Cerebral Circulation

In 2004, Taguchi et al reported that after a 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. Endothelial progenitor cells have angiogenic and vascular reparative capabilities that make them ideal for neurovascular repair.^{54,55} Such a rich vascular environment, along with the generation of other nurturing neuronal mediators from CD34+ cells, such as VEGF, epidermal growth factor 2, and insulin-like growth factor 1–1, enhances subsequent neuronal regeneration.^{56,57} 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.⁵⁸ In 2020, Kikuchi-Taura et al reported that angiogenesis is activated by BM-MNCs via gap junction-mediated cell–cell interaction and that cell–cell interaction via gap junction is the prominent pathway for activation of angiogenesis at endothelial cells after ischemia and provided novel paradigm that energy source supply by stem cell to injured cell is one of the therapeutic mechanisms of cell-based therapy.⁵⁹

Enhancement of the Regenerative Process by Neurogenesis

Neural stem/progenitor cells participate in the regenerative response to perinatal HI.⁶⁰ One report suggests that hematopoietic stem cells could differentiate into nonlymphohematopoietic cells such as neurons or microglia or could stimulate neurogenesis.⁶¹ However, it is uncertain whether this is significantly effective for neonates with HIE.^{62–65} We reported UC-MSCs could enhance neurogenesis with high expression of growth-associated protein 43 in injured neurons, and also confirmed elongated processes in injured neurons. We also previously showed that UC-MSCs exert their neuroprotective effects partially through secretion of BDNF and HGF by inhibiting the apoptosis/necrosis of injured neurons.³⁴

History of Clinical Therapies for HIE

History of TH and Cell Therapies for Neonatal HIE

The 2010 revised International Liaison Committee on Resuscitation guidelines⁶⁶ stated that infants born at or near

term with evolving moderate-to-severe HIE should be offered TH, based on three large-scale randomized controlled trials.

However, TH must be initiated within the first 6 hours after birth. TH showed protective effects against HIE mainly in acute stages. By contrast, cell therapy may have a much longer therapeutic time window over acute stages because it might reduce apoptosis/oxidative stress and enhance the regenerative process. Furthermore, cell therapies such as UCBCs and UC-MSCs are being incorporated into new protocols for protection against ischemic brain damage in some countries. Cotten et al reported autologous UCBC phase 1 clinical study for newborns with HIE for the first time in 2013.⁵⁵ Twenty-three infants were cooled and received cells. Median collection and infusion volumes were 36 and 4.3 mL. Vital signs including oxygen saturation were similar before and after infusions in the first 48 postnatal hours. Cell recipients and concurrent cooled infants had similar hospital outcomes (mortality, oral feeds at discharge). Thirteen of 18 (74%) cell recipients and 19 of 46 (41%) concurrent cooled infants with known 1 year outcomes survived with Bayley III scores ≥ 85 in three domains (cognitive, language, and motor development).⁶⁷ In 2014, we administered autologous UCBC therapy for neonatal HIE, for the first time in Japan.² 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. The enrollment criteria for our autologous UCBC study are the same as the inclusion/exclusion criteria for TH in Japan. 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. There were no serious adverse events that might be related to cell therapy in all six newborns. At 30 days of age, the six infants survived without circulatory or respiratory support. At 18 months of age, neurological development was normal in four infants and delayed in two infants. This study shows that autologous UCBC therapy is feasible and safe.⁶⁸

Recently, MSCs have been attracting much attention for their therapeutic potential for neurological disorders.⁶⁹ Huang et al administered UC-MSCs for CP and reported that UC-MSC infusion with basic rehabilitation was safe and effective in improving gross motor functions in children with CP.⁷⁰ On the contrary, the Nagamura-Mukai group focused on UC because of (1) abundance and ease of collection, (2) noninvasive collection, (3) little ethical controversy, (4) low immunogenicity with significant immunosuppressive ability, and (5) migration ability toward injured sites.^{36,45} We plan to use UC-MSCs for neonatal HIE or CP, after using them as a regenerative product for GVHD.

Clinical Trials Using UC-MSCs

Clinical trials using UC-MSCs for neurological disorders have been increasing in number and the recent clinical reports are summarized in [Table 2](#).

Table 2 Summary of recent clinical trials using UC-MSCs for neurological disorders (Modified from Mukai et al⁶⁹)

Reference	Disease	Number of patients	Mean age (range), y	Route of administration	Number of cells	Number of treatments	Results	Adverse events
Wang et al (2015) ⁷¹	CP	16 (8 twins)	6.29 (3–12)	IT	1–1.5 × 10 ⁷ cells at 3–5 days intervals	4	Motor functional recovery after 1 and 6 months	No
Dong et al (2018) ⁷²	CP	1	4	IT + IV × 2 IT × 1	5.3 × 10 ⁷ cells in total	3	Improvements in EEG and limb strength, motor function and language expression	No
Huang et al (2018) ⁷⁰	CP	27	7.3 (3–12)	IV	5.0 × 10 ⁷ cells	4 × 2 course	The changes in the total proportion of the GMFM-88 and total scores of CFA in the hUCB-MSC group were significantly higher than that in control group at 3, 6, 12, 24 months	No serious adverse events
Bonczkowski and Zdołńska-Malinowska (2019) ⁷³	CP	54	17m-17	IV	The maximum single dose 0.5 × 10 ⁶ –2.14 × 10 ⁶ cells/kg	1–10 injections	48 of 54 analyzed patients (88.9%) achieved some improvement in health status. Forty-eight (88.9%) patients experienced an increase in their QoL, and 21 patients (38.9%) achieved an increase in their self-sufficiency level	Epileptic seizures, emotional hypersensitivity, fever, headache, nausea, vomiting
Fu et al (2019) ⁷⁴	CP	57	1m-12	IT	4.0 or 8.0 × 10 ⁷ cells (4 times of 1.0 × 10 ⁷ cells in 1 course)	4 or 8 (1 or 2 courses)	Gross motor and fine motor function scores with one course of transplantation were significantly increased at 6 months. Another course of transplantation further improved gross and fine motor function	Dizziness, headache and fever
Gu et al (2020) ⁷⁵	CP	20	3.83 (2–12)	IV	4.5–5.5 × 10 ⁷ cells at 7 days intervals	4	Significant improvements in ADL, CFA, and GMFM were observed in the hUC-MSC group compared with the control group	No significant adverse event incidence.
Cheng H et al (2014) ⁷⁶	SCA	10	35.3 (19–57)	IT	2 × 10 ⁷ cells at 10 days intervals	2	Motor functional recovery (7/10) after 6 months	No
Jin et al (2013) ⁷⁷	SCA	16	39.9 (21–56)	IT + IV	IV: 4 and 2 × 10 ⁷ cells. IT: 2 × 10 ⁷ cells × 2 at 7 days intervals	4	Motor functional recovery after 6 months	No
Wang et al (2013) ⁷⁸	Traumatic brain injury	20	27.5 (5–48)	IT	10 ⁷ cells at 5–7 days intervals	4	Motor functional recovery after 6 months	No
Li et al (2014) ⁷⁹	MS	13	41.7	IV	4 × 10 ⁶ cells/kg every 2 weeks	3	The overall symptoms of the hUC-MSC patients improved compared with patients in the control group. Both the EDSS scores and relapse occurrence were significantly lower than those of the control patients	No
Riordan et al (2018) ⁸⁰	MS	20	41.15 (24–55)	IV	2 × 10 ⁷ cells /day	7	Improvements in EDSS scores, in bladder, bowel and sexual dysfunction, in nondominant hand average scores, in walk times and general perspective of a positive health change	No serious adverse events
Lv et al (2013) ⁸¹	Autism spectrum disorder	9	6.20(4.0–9.8)	IT + IV	1 × 10 ⁶ cells /kg at 5–7 days intervals	4	Statistically significant differences were shown on CARs, ABC scores and CGI evaluation in the treatment groups compared with the control at 24 weeks post-treatment (p < 0.05)	Epileptic seizures more frequent, emotional hypersensitivity, fever, headache, nausea, vomiting
Riordan et al (2019) ⁸²	Autism spectrum disorder	20	10.25 (6–15)	IV	9 × 10 ⁶ cells at 12 weeks intervals	4	ATEC and CARs scores of eight subjects decrease	No treatment-related serious adverse events

Abbreviations: ABC, aberrant behavior checklist; ADL, activities of daily living; ATEC, Autism Treatment Evaluation Checklist; CARs, The Childhood Autism Rating Scale; CGI, clinical global impression; CP, cerebral palsy; EDSS, Kurtzke Expanded Disability Status Scale; EEG, electroencephalography; GMFM88, gross motor function measurement 88; hUCB-MSC, human umbilical cord blood-derived mesenchymal stem cells; hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; IT, intrathecal injection; IV, intravenous injection; MS, multiple sclerosis; QoL, quality of life; SCA, spinocerebellar ataxia.

Recent clinical trials using UC-MSCs cover a wide range of neurological diseases including CP ($n = 6$), spinal cord injury ($n = 1$), spinocerebellar ataxia ($n = 1$), traumatic brain injury ($n = 1$), multiple sclerosis ($n = 2$), and autism spectrum disorder ($n = 2$). Most studies were performed using intrathecal (IT) ($n = 5$), intravenous (IV) ($n = 6$), and IT + IV ($n = 2$) injection, and multiple administration of cells: two times ($n = 1$), three times ($n = 2$), four times ($n = 6$), seven times ($n = 1$), eight times ($n = 1$), four or eight times ($n = 1$), and others ($n = 1$). The number of cells of administration is various (each dose 9×10^6 – 8×10^7 cells or 0.5 – 4.0×10^6 cells/kg). IV injection is easier compared with IT, but UC-MSCs are surely distributed to the central nervous system without being trapped in the lung and blood–brain barrier in IT injection

Autologous transplantation is required to avoid transplant rejection, but timing is crucial. UC-MSCs preparation takes 3 months or more and to confirm their quality (from infection and genetic testing), it is impossible to administer autologous UC-MSCs in the acute phase after neurological injuries. Also, establishment of a system in cryopreservation of autologous UC is required. Nonetheless, allogeneic MSCs can be ordered as a preparation anytime and administered in the acute to subacute phase.

Considering UC-MSC therapy for HIE, administration of UC-MSCs in the acute phase might be expected with the aim of suppressing the commencement of brain damage cascade. Therefore, UC-MSCs used for HIE should be allogeneic and should be prepared immediately. UC-MSC therapy for CP is performed considering neurotrophic effects of UC-MSCs in addition to immunomodulation against chronic inflammation. Hence, UC-MSCs used for CP could be both autologous and allogeneic.

Few serious adverse events were observed after transplantation, and most reports suggest that UC-MSC has a therapeutic potential with relative safety. MSCs for neurological diseases are expected as a new cell therapy by combining with rehabilitation and other medication therapies suggested by recent clinical trials.

Conclusion

Since the establishment of consensus suggesting that TH should be offered for newborn HIE, it is still challenging to prevent brain damage from complicated cascade of HIE. We suggest that the next step will include reaching a consensus on cell therapies for HIE and establishment of effective protocols for cell therapy for HIE.

Authors' Contributions

M.N. and H.S. conceptualized the manuscript; T.M. was involved in methodology and software; T.M. and H.S. were involved in validation. M.N. wrote the original draft and was involved in formal analysis and investigation, and manuscript preparation, review, and editing. H.S. was involved in supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

None declared.

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