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Review Article

Neonatal Hypoxic Ischemic Encephalopathy: An Updated Preclinical and Clinical Review

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Abstract

Hypoxic-ischemic encephalopathy is a major cause of death and disability in the newborn period. Experimental models have demonstrated that brain injury in hypoxic-ischemic encephalopathy occurs in two phases: primary energy failure and secondary energy failure. In primary energy failure, there is a cessation of oxidative metabolism and irreversible neuronal injury, followed by secondary energy failure due to glutamate release, elevation of intracellular calcium, reactive oxygen species formation, apoptotic cell death, and activation of the immune system. Secondary energy failure is the target of therapeutic intervention in neonatal hypoxic-ischemic encephalopathy. The sensitivities of cell types to hypoxic injury leads to the differences in injury pattern seen in term versus preterm infants. Current treatment recommendations include identification and treatment of seizures when present and prompt administration of therapeutic hypothermia if clinical parameters are met. This paper also discusses new treatment paradigms being developed, including erythropoietin, and the role of neuroimaging as a predictive tool for neurodevelopmental outcome.

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Keywords
Hypoxic Ischemic Encephalopathy; HIE; neonatal neurology; therapeutic hypothermia; erythropoietin

1. Introduction

Hypoxic-ischemic encephalopathy (HIE) occurs in 1 to 6 per 1,000 live term births and is associated with considerable morbidity and mortality in the neonate [1]. In developing countries the prevalence of birth asphyxia and HIE is much higher, contributing to disproportionate morbidity and mortality in these areas [2]. HIE is a subtype of neonatal encephalopathy, which is a clinically defined syndrome in late preterm or term neonates that have symptoms of neurologic dysfunction, manifested by decreased levels of consciousness, seizures, and often respiratory difficulties. Detrimental effects on neurodevelopment occur more commonly in neonates with moderate to severe forms of encephalopathy compared to infants with mild HIE. In 1976, Sarnat and Sarnat developed descriptive staging based on examination to classify infants from mild to severe encephalopathy. More recent trials have used a modified Sarnat staging to exclude the heart rate variability that occurs with HIE treatment [3, 4]. Sarnat stages are used as a predictor of long-term prognosis and as inclusion criteria for therapeutic hypothermia. Infants with moderate encephalopathy, or Stage II, demonstrate lethargy with decreased spontaneous activity, abnormal EEG with frequent discharges and seizures, and often hypotonia and autonomic dysfunction. In Sarnat Stage III, or severe encephalopathy, stupor or coma is the major presenting symptom at birth, with profoundly low tone, absent primitive reflexes and an abnormal EEG, with severe attenuation and infrequent discharges [3].

2. Pathophysiology of HIE

Given the prevalence and severity of outcomes as described above, much research has been done to understand the pathophysiology of neonatal HIE. Using various animal and cellular models as well as human tissue samples, there has been significant progress in understanding pathogenesis of motor and cognitive deficits seen in affected infants. Perinatal hypoxic ischemic injury causes both acute and chronic changes to the developing brain. In this section, the cellular cascade of the progression from initial acute energy failure through secondary energy failure will be explored as well the mechanistic rationale for current treatments of HIE. Compared to the adult brain, the developing brain is uniquely susceptible to hypoxic injuries. Neonatal brains have more fragile and immature microvasculature without a population of neighboring astrocytes for vascular support [5]. The neonatal brain has decreased capacity for perfusion autoregulation than the adult brain [6]. Finally, immature neurons and progenitor populations have increased sensitivity to hypoxic events compared to fully differentiated neuronal populations in the adult [7, 8].

Histologically, the progression of neuronal injury has been well described in animal models. With hypoxic ischemic injury, there is an acute primary energy failure with cessation of oxidative metabolism followed by a secondary energy failure due to downstream inflammation, excitotoxic changes and oxidative stress. In rodents, within 10 minutes of hypoxia, neuronal injury is

evidenced by mitochondrial swelling [9, 10]. This progresses within 48 hours of the initial injury, with development of eosinophilic cytoplasm, pyknotic nuclei and cytoplasmic swelling [10].

Following a hypoxic ischemic injury to the developing brain, there is an acute primary energy failure initiated by the loss of oxidative metabolism resulting in ATP depletion and lactate accumulation. ATP depletion subsequently causes failure of ATP-dependent sodium/potassium pumps and calcium pumps, leading to increasing intracellular sodium and calcium concentrations, water influx and cellular swelling [11]. Brain MRI can detect this cellular swelling in less than an hour of the hypoxic insult in animal models of HIE [12]. During this phase of primary energy failure, cells that were most severely affected by the hypoxic conditions may swell and necrose, resulting in primary neuronal cell death [13].

Cells that do not undergo primary cell death via necrosis begin a cascade towards secondary injury, due to downstream excitotoxicity, free radical production, oxidative stress, inflammation and the apoptotic cascade. The progression from primary energy failure to secondary energy failure has been demonstrated in both animal models and neonates using MR-spectroscopy 6-48 hours after the initial injury (Figure 1) [14, 15].

Excitotoxity, in particular, is a major driver of secondary injury after primary energy failure in HIE. Glutamate is the major excitatory neurotransmitter in the brain and most neurons have glutamate receptors. Increased intracellular calcium from primary energy failure results in active release of glutamate into the extracellular space, adding to glutamate released by necrosis of glutamatergic neurons. As demonstrated by animal models of HIE, increased glutamate release coupled with decreased active uptake of glutamate by astrocytes in the injured brain results in significant increases in extracellular glutamate [16, 17]. Increased extracellular glutamate saturates N-methyl-d-aspartate (NMDA) receptors, driving more calcium into neurons [18].

The excess influx of calcium into the cell drives a number of subsequent damaging processes. Increased calcium both activates nitric oxide synthase activity and induces increased expression of the gene, thus increasing nitric oxide production [19, 20]. Nitric oxide and other free radicals are highly reactive with many cellular components. The neonatal brain contains high levels of unsaturated fatty acids, which are particularly sensitive to free radical damage, and reduced production of endogenous antioxidants. These two factors make the neonatal brain particularly vulnerable to free radical and oxidative injury [21, 22]. In addition to the structural damage to the cellular membrane and cellular organelles, free radicals also contribute to the apoptotic cascade.

The inflammatory process after hypoxic injury lasts days and furthers neuronal injury by increasing extracellular glutamate and free radical species, as well as activating apoptotic cascades. Both cell rupture from necrotic cell death during the primary energy failure and damage caused by free radical species in damaged but not necrosed cells cause a local inflammatory response. Activated astrocytes and damaged endothelial cells release cytokines recruiting microglia and leukocytes to the injury [23, 24]. Microglia begin to accumulate within hours of the initial injury and increase over the next few days [25]. Microglia release nitric oxide and glutamate propagating the injuries described above [26]. In addition, cytokines themselves can activate nitric oxide synthase as well as contribute to the pro-apoptotic cascade [27].

The combination of the primary energy failure with mitochondrial dysfunction, excitotoxicity, free radical production, and inflammation all converge on pro-apoptotic pathways resulting in delayed neuronal death observed days after the initial insult. Mitochondrial damage from oxidative stress results in release of cytochrome c, a pro-apoptotic factor into the cytoplasm [28].

Additionally, cytokines can signal through various tumor necrosis factor family receptors also initiating apoptosis through separate pathways [29]. The process from initiation of this pathway within hours of the hypoxic injury extends over many hours to days before resulting in programmed cell death [30]. It is important to note that unlike necrosis, apoptosis is an active process that requires significant energy production to complete, so it is dependent on reperfusion and return of ATP stores following resumption of oxidative metabolism.

This progression from the immediate primary energy failure to late downstream secondary mechanisms of injury are well described in neurons. In contrast to the well-defined chronology of injury in neurons following a period of hypoxia, astrocytes and oligodendrocytes seem relatively resistant to further injury. In animal models, the rapid histological change in neurons within minutes is contrasted with no histological alterations in astrocyte cells [31]. Additionally, while immature oligodendrocytes behave similarly to neurons with hypoxic insult, mature oligodendrocytes are relatively resistant [32]. The sensitivity of neurons and immature oligodendrocytes compared to astrocytes and fully differentiated oligodendrocytes partially explains the typical patterns of HIE seen in infants. In premature infants, white matter predominant injuries are most common, due to the higher population of immature oligodendrocytes. In contrast, term infants have more gray matter injury in response to hypoxia, due to fewer immature oligodendrocytes present in the term brain and relative resistance of mature oligodendrocytes to hypoxia [32]. Regional differences in neurochemistry also helps to explain the patterns typically seen on brain imaging in neonatal HIE. For instance, the basal ganglia in the newborn brain may be uniquely sensitive to hypoxic injury due to a combination of (1) increased metabolic demand, (2) high concentrations of nitric oxide synthase making these areas sensitive to oxidative stress, and (3) subtypes of immature NMDA receptors sensitive to prolonged opening and excitotoxicity [33-35].

3. Treatment of HIE

3.1 Rationale

Ideally, therapeutic strategies should target preventing both primary and secondary energy failures. However, given the time needed to identify HIE and mobilize for therapeutic intervention, more therapeutic strategies have targeted the mechanisms in the latent period between primary energy failure and the secondary energy failure that occurs hours to days later.

Therapeutic hypothermia, which the majority of research and clinical emphasis has been placed, targets the mechanisms behind secondary injury in multiple ways. Most obviously, lowering brain temperatures decreases metabolic rate and therefore decreases potential perfusion metabolism mismatch. With decreased ongoing primary energy failure, propagation of the initial injury is minimized [36]. Additionally, hypothermia reduces extracellular glutamate and free radical production following hypoxia [37]. Hypothermia also inhibits activation of caspase-3, an important component of the apoptotic cascade downstream of both cytokine and cytochrome c activation in the pathway [38]. By targeting excitotoxicity, free radical production, and apoptosis simultaneously, much work has been done showing the clinical benefits to therapeutic hypothermia as will be described later.

There are many other promising mechanistic targets currently being explored. Excitotoxicity has been targeted at many levels. The use of anticonvulsants such as lamotrigine and topiramate to decrease glutamate release has been proposed [39, 40]. Additionally, NMDA receptor inhibitors including memantine and magnesium have also been studied [41-43]. Melatonin has been shown to be a powerful antioxidant, reducing the effects of free radicals on secondary injury, and N-Acetyl cysteine has also been proposed as pharmacologic means to reduce oxidative stress [44, 45]. Finally, erythropoietin has been proposed to act on many levels of secondary injury, inhibiting excitotoxicity, reducing free radicals, and inhibit downstream apoptotic signaling [46]. There are many exciting mechanistic targets for treating HIE during the latent phase before secondary injury, but currently therapeutic hypothermia remains the gold standard treatment.

3.2 Current Standard Treatment

Therapeutic hypothermia is a safe and effective treatment for moderate or severe neonatal encephalopathy as set forth by the American Academy of Pediatrics [47]. This standard of care is based on randomized clinical trials that demonstrated improvement in neurodevelopment at ≥18 months of cooled infants compared to controls, in infants at least 35 weeks' gestation at birth and weighing greater than 1800 grams (Table 1) [48-51]. In a meta-analysis of 11 randomized controlled trials, therapeutic hypothermia reduced the composite outcome of mortality or neurodevelopmental disability at 18 to 24 months of age, with a relative risk reduction of 25% [52]. The number needed to treat for one additional patient to benefit is 6 neonates with moderate encephalopathy and 7 with severe encephalopathy [52].

As described in detail above, therapeutic hypothermia is thought to reduce cerebral metabolism and lower the temperature of vulnerable deep structures such as the basal ganglia. In addition to minimal gestational age, weight, and evidence of Sarnat stage II or III encephalopathy on exam within 6 hours of birth, infants are selected for therapeutic hypothermia based upon: severe acidosis on first blood or cord gas (pH <7 or base deficit >16 mmol/L), or Apgar score ≤5 in 10 minutes, or prolonged resuscitation [48, 49].

The cooling must be initiated within 6 hours of life. Cooling devices are often set to a target temperature of 33.5°C (range 33.5°C to 34.5°C), using an esophageal or a rectal temperature probe. Neonates are cooled for 72 hours, then slowly rewarmed over 6 hours (0.5°C/hour) to a target of 36.5 °C. Adverse effects of hypothermia include sinus bradycardia, prolonged QT interval, skin reddening or hardening, and rarely, subcutaneous fat necrosis. Thrombocytopenia and other coagulopathies, sepsis, and pneumonia are reported as well.

As cooling modalities (head versus body) differ between medical centers, the clinical efficacy and safety of selective head cooling (SHC) compared to whole body cooling (WBC) has been further investigated [53]. In WBC, the rectal temperature is maintained between 33 to 34°C, compared to a core temperature of 34 to 35°C in SHC. In regards to short-term outcomes, including days of hospitalization, need for tracheostomy or gastrostomy tube at discharge, rates of thrombocytopenia and mortality rates, studies have demonstrated no significant differences between cooling types [53, 54]. There have been no reported differences in long-term neurologic outcomes between the 2 methods on meta-analysis, however many centers favor WBC due to ease of use, cost-effectiveness, and to minimize scalp breakdown and EEG interference.

Multiple studies have investigated and continue to explore expanded windows for cooling (>6 hours of age), degree of cooling (<33^{o}C), duration of therapy (>72 hours) and effect on preterm gestation (ages 32 to 35 weeks) or those with mild encephalopathy (Sarnat stage I) [55-60]. These studies could potentially benefit neonates born at community hospitals who require longer transportation time to a center with cooling capabilities, and late preterm infants.

3.3 Management of Seizures Secondary to HIE

Neonatal seizures are a common symptom of hypoxic brain injury, though do not always predict a worse outcome [61]. Seizures may be clinically silent, subtle (e.g. abnormal eye movements, tongue or lip smacking, bicycling of arms or legs, or apneic episodes) or focal with clonic movements [1]. Neonatal seizures typically occur within the first 48 hours after insult. Earlier seizure onset, less than 6 hours of age, is suspicious for an in-utero insult. Therapeutic hypothermia may reduce the frequency and duration of seizures in moderate, but not severe, HIE [61]. Standard electroencephalography (EEG) is needed for appropriate diagnosis and management of neonatal seizures secondary to HIE, during cooling, rewarming and post-rewarming stages [62]. In a randomized control trial that compared treatment of only clinical seizures versus all electrographic seizures in HIE, authors found a significant association between seizure burden and worse injury on brain MRI [62]. Infants who received treatment for only clinical seizures had a higher seizure burden later in life than those that all electrographic seizures were treated. Thus continued electrographic seizures are associated with worsened neuroimaging and neurodevelopmental outcomes at 18 to 24 months. The anti-epileptic drug of choice for neonatal seizures is both clinician- and center-dependent, but the most frequently used first-line medications at this age are phenobarbital and levetiracetam. Each medication only controls seizures in 50% of neonates [61]. Phenobarbital use during the neonatal period compared to levetiracetam, however, has been associated with neuronal apoptosis in animal models and reduced cognitive and motor scores at 2 years of age in children [63]. In general, anticonvulsants are required during this acute symptomatic phase, 1 week after injury, and can often be discontinued early.

4. Emerging Management of Neonatal HIE

4.1 Serum Biomarkers

Several biomarkers have been investigated to aid in the prognostication for neonates with HIE, including S100-B, ubiquitin carboxyl-terminal esterase L1, glial fibrillary acidic protein, creatine kinase brain band, neuron-specific enolase (NSE), and malondialdehyde [61, 64]. S100-B is expressed by astrocytes, and elevated levels in serum and cerebrospinal fluid have been used as biomarkers for brain injury [65]. Elevated NSE levels are also associated with brain injury in HIE [66, 67]. New approaches have identified haptoglobin and S100A8 as potential markers of HIE severity but will need to be further validated [68]. Additionally, brain derived neurotrophic factor (BDNF) and tau have been linked to neurodevelopmental outcomes [69]. An ongoing study is currently exploring interleukin-6, metalloproteinase-9, tissue inhibitor of metalloproteinases-1 (TIMP-1), albumin modified by hypoxia, troponin I, acylcarnitines and amino acids as prognostic

factors [70]. While exciting, this work towards a robust and reliable biomarker for HIE severity and prognostication still needs much research before wide clinical use.

4.2 Adjuvant Treatments

Multiple neuroprotective therapies have been studied as adjuvant treatment in neonatal HIE, and many have ongoing clinical trials. These medications include antiepileptic medications (topiramate), memantine, erythropoietin (Epo), darbepoietin, magnesium, melatonin, clonidine, vitamin D, caffeine, allopurinol and stem cell therapy [47, 64, 71-79].

Of the adjuvant therapies listed, Epo appears to be medication closest to clinical use. There is a currently a Phase III trial investigating the efficacy of high dose erythropoietin in conjunction with hypothermia for neuroprotection in infants with HIE after promising results in phase I/II trials [80-82]. In the Phase II, double-blinded, placebo-controlled trial by Wu, et al., high dose Epo (1000 U/kg) was given intravenously to infants for 5 days (at day of life 1, 2, 3, 5, and 7) in addition to therapeutic hypothermia. The authors found that high dose Epo with hypothermia reduced the extent of brain injury on brain MRI compared to those treated with TH alone, and cooled infants had improved motor outcomes at 1 year of age. Side effects seen in the trial were not thought to be related to Epo, which included deep vein thrombosis (1 patient in each group) and cardiac compressions and intubation in the setting of severe multiorgan injury. A recent case-control study looked at the use of Vitamin D as adjuvant treatment for neonates with Sarnat grade II HIE, who were also treated with erythropoietin and magnesium sulfate, and found neonates treated with Vitamin D had reduced serum S100-B calcium-binding protein (S100-B) levels [83]. There are also studies assessing the benefit of magnesium sulfate and melatonin in neonatal HIE, using S100-B as a biomarker [74].

There are Phase I trials investigating the use of stem cells from bone marrow or umbilical cord, which may provide a protective effect on the neonatal brain after injury [84]. While hypothermia has a narrow therapeutic window, stem cells may be able to prevent inflammation and neuronal injury and regenerate tissue over a longer period.

While some therapies have promise, others like xenon and cannabidiol have failed to show therapeutic value [85, 86]. Other therapies' benefits remain unknown, particularly magnesium. Randomized controlled trials have demonstrated the neuroprotective effects of antenatal magnesium sulfate in preterm infants, specifically in reducing the risk of fetal death or cerebral palsy [87]. However, other studies have found no differences in short-term outcomes if infants are treated with magnesium [88].

4.3 Neuroimaging as a Predictive Tool

Brain magnetic resonance imaging (MRI) to detect injury is recommended between days of life 5 (after hypothermia) and 14, and is most useful as a predictive tool in neonates with moderate and severe encephalopathy [64, 89, 90]. Imaging obtained too early (i.e. from the first hours after injury and up to 3 days) may underestimate the extent of injury, whereas imaging obtained after days 8 to 10 may have resolution of diffuse weighted imaging (DWI) findings and be falsely reassuring [61, 90]. T1 and T2 MRI sequences within the first 2 weeks of life is of greater prognostic utility than DWI with apparent diffusion coefficient, as there is a 98% sensitivity and 76% specificity in T1 and T2 for determining long-term outcomes [61]. Abnormally bright signal or

hyperintensity on DWI and T1 weighted images in this clinical setting is consistent with anoxic brain injury, most commonly seen in the basal ganglia and thalami in infants with suspected HIE, and at times the posterior limb of the internal capsule [90]. Three patterns of damage after ischemia are recognized on neonatal MRI: (1) injury to the thalami and/or putamen with subcortical white matter involvement: (2) injury to the parasagittal gray matter and subcortical white matter; and (3) focal or multifocal injury.

Magnetic resonance spectroscopy (MRS) has increasingly been used as a quantitative biomarker of brain injury and should be utilized if available [1, 64]. This brief imaging technique analyzes metabolites in specific areas of the brain, often the basal ganglia in neonates with suspected HIE, including lactate, *N*-acetyl aspartate (NAA), choline and creatine. Elevated lactate/NAA or lactate/choline ratios, or lactate/creatine ratios >1.0 ratios in the first 2 postnatal weeks portend long-term neurodevelopmental disability [90].

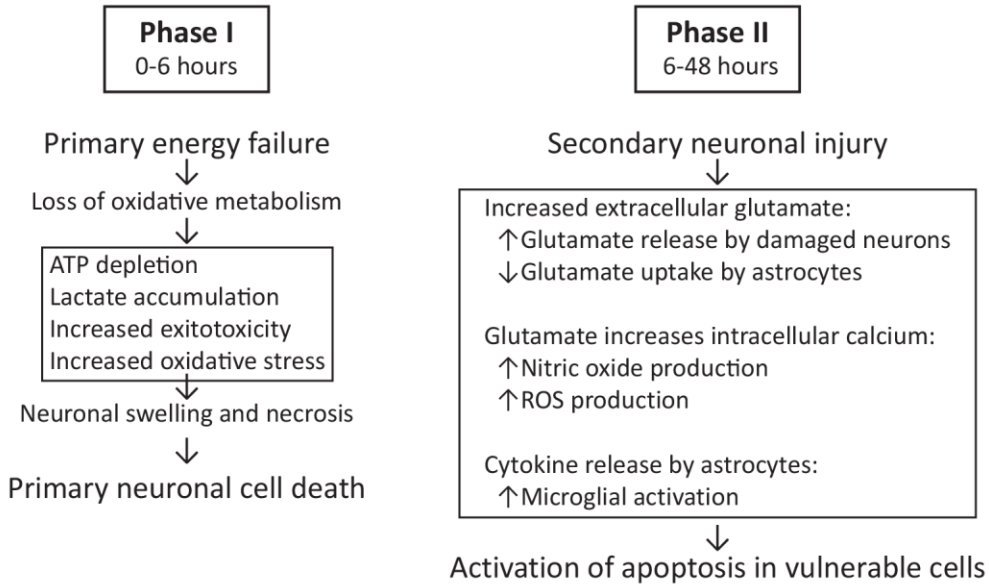

Figure 1 The initial phases of injury in HIE.

Table 1 Summary of the Outcomes of Major Randomized Clinical Trials on Therapeutic Hypothermia for HIE.

First Author, year	Type of Cooling	Primary Outcome	Secondary Outcomes
		Death or moderate or severe disability at ≥18mo, n cooled (%) vs n control (%), p value	
Shankaran, 2005	Whole body	45 (44%) vs 64 (62%), p=0.01	No major differences in rate of cerebral palsy
Gluckman, 2005	Head cooling, with mild systemic hypothermia	59 (55%) vs 73 (66%), p=0.1	Cooling improved survival in infants with less severe aEEG changes (p=0.009)
Azzopardi, 2009	Whole body	74 (45%) vs 86 (53%), RR 0.86,	Cooling increased rates of

	p=0.17	survival without neurologic abnormality (p=0.003); Cooling decreased rates of cerebral palsy (p=0.03)
Simbruner, 2010 Whole body	27 (51%) vs 48 (83%), p=0.001	Cooling had a protective effect in the group with severe HIE (p=0.005)

Controls: intensive care alone; RR: relative risk

5. Conclusions

In summary, neonatal HIE confers significant morbidity and mortality. Brain injury in neonatal HIE is caused by at least two processes: a primary energy failure leading to neuronal and glial cell loss, and a secondary energy failure from a cascade of insults including excitotoxic injury from extracellular glutamate, inflammation, and oxidative damage. At this point, there are no effective treatments for primary energy failure in neonatal HIE but secondary energy failure has proven amenable to therapeutic intervention, as head- and whole body- cooling trials have shown. A greater understanding of the pathophysiology of the secondary process has opened the door to new treatments for neonatal HIE, several of which have shown promise. Topiramate, erythropoietin, melatonin, clonidine, vitamin D, and stem cell therapy are all currently being investigated for a role in the treatment of neonatal HIE. In addition to the proven benefit of therapeutic hypothermia, there is hope that these compounds may further augment whole-body or head cooling in the treatment of neonates with HIE.

Author Contributions

Concept and design: E. A. Armour, A. M. Curcio, R. H. Fryer. Literature Review: E. A. Armour, A. M. Curcio. Drafting of the manuscript: E. A. Armour, A. M. Curcio. Critical revision of the manuscript for important intellectual content: E. A. Armour, A. M. Curcio, R. H. Fryer.

Competing Interests

The authors have declared that no competing interests exist.

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