Uncovering the Role of Inflammation with Asphyxia in the Newborn

Simerdeep K. Dhillon, PhD[a], Pierre Gressens, MD, PhD[b], John Barks, MD, PhD[c], Alistair J. Gunn, MBChB, PhD[a,*]

KEYWORDS

• Hypoxia-ischemia • Inflammation • Anti-inflammatory treatments

KEY POINTS

- Acute and chronic inflammation triggered after hypoxia-ischemia (HI) is a critical mediator of secondary cell loss and subsequent injury and repair during the tertiary phase.
- HI can co-occur with perinatal inflammation or infection, and modulation of the immune responses to HI by pre-existing inflammation can be associated with either exacerbation or attenuation of neural injury.
- There is mixed clinical and preclinical evidence for the reduced efficacy of therapeutic hypothermia in the presence of perinatal infection/inflammation.
- Recent preclinical data suggest that targeting acute inflammatory processes after HI may be a viable strategy for treating inflammation-sensitized HI.

INTRODUCTION

Hypoxia-ischemia (HI) before or at birth is the single most common cause of perinatal brain injury in term infants, affecting approximately 1.5 to 3/1000 live births annually in high-income countries, leading to approximately 1 million neonatal deaths and life-long disabilities in survivors around the world.[1] Exposure to HI is also a significant contributor to the multifactorial etiology of preterm brain damage. A large cohort study reported that rates of hypoxic-ischemic encephalopathy (HIE) in preterm infants were significantly higher than at term, with moderate–severe HIE in 37.3/1000 preterm-born infants.[2]

Other etiologies associated with neonatal encephalopathy include perinatal infections, placental pathologies, and maternal comorbidities.[3] The amniotic cavity is maintained sterile in a healthy pregnancy. Pathogens can invade the amniotic cavity via

[a] Department of Physiology, The University of Auckland, 85 Park Road, Grafton, Auckland 1023, New Zealand; [b] Université Paris Cité, NeuroDiderot, Inserm, F-75019 Paris, France; [c] Department of Pediatrics and Communicable Diseases, The University of Michigan, 2018 MLB, Ann Arbor, MI 48109, USA
* Corresponding author.
E-mail address: aj.gunn@auckland.ac.nz

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placental transfer from maternal blood or ascend from the lower genital tract, causing intra-amniotic infection and fetal inflammation and infection.[4] Intrauterine inflammation can also be induced in the absence of microbes by endogenous molecules associated with cellular stress and damage.[5] Intra-amniotic infection or inflammation induces an inflammatory response in the fetal tissues in direct contact with amniotic fluid and subsequently can lead to a robust systemic and cerebral inflammatory response and associated brain injury.[6]

Retrospective cohort studies have reported inflammatory placental pathology and fetal inflammation in approximately 43% of term-born infants, in the absence of evidence for sentinel events. Moreover, histologic funisitis was an independent risk factor for neonatal encephalopathy.[7] Histologic chorioamnionitis is reported in nearly 95% of preterm births at 21 to 24 weeks of gestation and in about 10% of deliveries at 33 to 36 weeks and is associated with an increased risk of MRI abnormalities at term equivalent age and delayed brain maturation.[8,9] Babies can also be exposed to infectious microorganisms during birth or postnatally. Early-onset sepsis occurs within 72 hours after birth and is associated with microorganisms acquired in utero or during birth; its incidence is reported as 0.56 to 0.79 per 1000 live births in term-born infants and 13.5 per 1000 in very preterm infants.[10,11] In turn, neonatal sepsis is associated with an increased risk of developing brain injury and adverse neurodevelopmental outcomes.[12]

Importantly, these inflammatory insults can also occur in combination with HI and have a cumulative contribution to the pathogenesis of perinatal brain injury. For example, compared with the population, a higher incidence of early neonatal infections is reported in term infants with HIE.[13] Chorioamnionitis and fetal vasculitis are also commonly observed in term infants with perinatal asphyxia.[3] In a subset of the high-dose erythropoietin for asphyxia and encephalopathy trial participants, nearly 40% (124 of 321) had evidence of histologic chorioamnionitis.[14] These placental pathologies are associated with acute electroencephalographic (EEG) abnormalities, injury severity on MRI, and adverse neurodevelopmental outcomes in term infants treated with therapeutic hypothermia.[15,16]

To change outcomes, we need to improve our understanding of the pathogenesis of perinatal brain injury and the complex interactions between different etiologies. Inflammation is the common mediator of neural injury for multiple perinatal insults.[17] This review discusses the role of acute and chronic inflammation in mediating neural injury after HI, modification of neuro-inflammatory responses with a combination of HI and inflammatory insults and potential interventions.

Acute Inflammatory Response After Hypoxia-Ischemia

HI events trigger a cascade of inflammatory processes, which play a crucial role in the evolution of injury over weeks. Reperfusion after HI is typically associated with transient recovery of mitochondrial function and cerebral metabolism during the latent phase. Despite no reduction in cerebral perfusion, this transient recovery can be followed by secondary deterioration of oxidative metabolism from 6 to 8 hours and ultimately bulk cell loss.[18] The acute pro-inflammatory processes during the latent phase have been shown to contribute to the progression of secondary deterioration and cell loss (**Fig. 1**).

Microglia are the resident immune-responsive cells. They are involved in immune surveillance, synaptic pruning, neuronal hemostasis, and establishing network connectivity in the developing brain.[19] Exposure of the microglial pattern recognition receptors to damage-associated molecules released after HI, leads to diffuse microglia activation.[20] "Activated" microglia undergo morphologic and functional

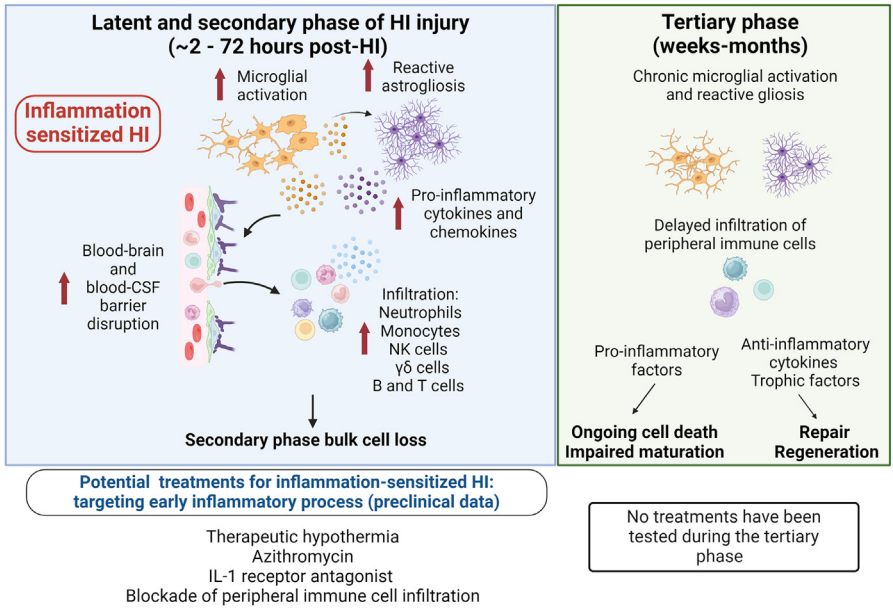

Fig. 1. Flow diagram showing inflammatory processes during the latent, secondary, and tertiary phases of HI injury, modulation of early inflammatory processes with pre-existing inflammatory challenge and potential neuroprotective treatments for inflammation-sensitized HI. Created with BioRender.com

changes, including upregulation of toll-like receptors expression, initiation of phagocytosis, and increased production of effector molecules like reactive oxygen species and inflammatory cytokines, and these inflammatory mediators can directly activate extrinsic cell death pathways.[19] It is important to appreciate that microglial activation is complex and encompasses multiple states; thus, this term should be considered to be short-hand for a highly polymorphic reality.[21]

Intense microglial activation and upregulation of pro-inflammatory cytokines like tumor necrosis factor (TNF), interleukin (IL)-1β and IL-6 have been observed as early as 2 to 3 hours after HI in preclinical studies.[22] The degree of acute neuro-inflammatory response was associated with neuronal loss within 24 hours after HI, and attenuation of the excessive early neuro-inflammation is neuroprotective.[22] In contrast, in neonatal mice, complete depletion of microglia significantly exacerbated brain injury at 3 days after HI, highlighting an endogenous neuroprotective role of acute microglial activation.[23] Consequently, the field has tended to focus on the functional categorization of microglia into M1 (activated: pro-inflammatory) and M2 (anti-inflammatory) phenotypes after HI and targeting induction of M2 phenotype for neuroprotection.[19] However, it is now recognized that such phenotypic classification may overly simplify the complexity of dynamic changes in microglia that are intricately associated with their local environment changes.[21] Understanding the temporal pattern of diverse microglial transcriptional changes after neonatal HI will aid the development of treatment strategies.

Astrocytes are important in maintaining homeostasis and a stable environment for normal neuronal function. Astrocytes respond to HI with morphologic changes such as hypertrophy of the cell body and processes, increased intermediate filaments, and changes in gene and protein expression.[24] Astrocytes can contribute to the

evolution of injury by producing inflammatory cytokines, disrupting trophic support to neurons and glia and propagating injury to the previously undamaged areas.[25] Studies in near-term fetal sheep have provided evidence that opening of connexin 43 hemichannel (the predominant astrocytic connexin) during the latent phase of recovery significantly contributes to spreading neural injury and increases seizure burden.[26] ATP and other neuroactive molecules released from hemichannels can act as damage-associated molecules to activate inflammatory pathways.[25] In addition, the opening of hemichannels may increase calcium influx, and in turn increased intracellular calcium can contribute to neuronal and oligodendrocyte death.[25] Like microglia, multiple astrocytic activation states and phenotypic plasticity are also being recognized.[27]

In addition to resident immune cell activation, HI is associated with a profound systemic inflammatory response. Neonates with HIE have increased peripheral leukocytes and elevated plasma concentrations of proinflammatory cytokines on the first day of life, which are associated with injury severity and adverse neurodevelopmental outcomes.[28] Peripheral immune cell activation and infiltration into the brain also plays a significant role in acute neural damage after HI. Recruitment of peripheral immune cells into the brain is facilitated by chemokine upregulation and transient disruption and increased permeability of the blood–brain barrier after HI[29]; in addition, alterations of the blood–cerebrospinal fluid barrier may facilitate immune cell infiltration.[30]

Studies in neonatal rodents have examined the temporal profile of peripheral immune cell recruitment into the brain after HI. In P9 mice, circulating neutrophils in the blood increased 12 hours post-HI, followed by infiltration into the brain, with cerebral neutrophil counts peaking at 24 hours, and then declining by 72 hours post-HI.[31] Similarly, other preclinical studies have shown waves of cerebral infiltration of peripheral monocytes, gamma-delta-T cells, natural killer cells, and peripheral B and T cells after neonatal HI.[32,33] More importantly, early depletion of peripheral immune cells in different models was consistently neuroprotective.[31,32] These data denote that acute peripheral immune cell infiltration contributes to the secondary phase of damage after HI. However, the precise time course of expression of different cells and their role in injury evolution needs further investigation. Peripheral immune cells can exacerbate neuro-inflammatory response by promoting glial activation, formation of neutrophil extracellular traps, and increased production of reactive oxygen species and pro-inflammatory cytokines.[34]

Tertiary Phase: Chronic Inflammation

The secondary phase of cell death lasts approximately 72 hours after severe HI. It resolves into a tertiary phase involving both ongoing cell death and repair and reorganization.[18] Serial imaging studies in term neonates with HIE have reported that subtle abnormalities in the regional signal intensity seen in the first few days after birth become more apparent by the end of the first week, and an MRI scan after 1 month can show gross changes such as volume loss, cysts, gliosis, and impaired myelination.[35] Similarly, preclinical studies have shown delayed evolution of injury over weeks after HI.[36] There is limited understanding of the mechanisms of this delayed evolution of injury, but there is evidence to support the role of chronic neuroinflammation.

In preterm fetal sheep exposed to severe HI, diffuse white matter loss and maturational arrest at 3 to 7 days post-HI evolve into severe cystic injury and white matter atrophy over 2 to 3 weeks.[37] The development of these cystic lesions in fetal sheep was preceded by intense local microgliosis. Treatment with a TNF inhibitor during the tertiary phase attenuated cystic white matter damage and improved neurorepair, supporting the concept that inflammation is a key mediator of the delayed evolution of injury.[38] Region-dependent, biphasic microglial activation over 2 weeks after

HI was also observed in neonatal mice.[33] In term-equivalent fetal sheep, neuronal loss, white matter damage, development of lesions, and EEG dysfunction at 7 days after 30 minutes cerebral ischemia were associated with persistent microgliosis and astrogliosis and upregulation for pro-inflammatory microglia.[39] Altered microenvironment with chronic microglial activation and reactive gliosis in the tertiary phase also contributes to impaired maturational processes, leading to microstructural abnormalities.[40]

Beneficial Effects of Microglia

Along with detrimental effects, microglia activation may also play a role in tissue remodeling and neurorepair. Based on activation of specific cell surface receptors, microglia phagocytose cellular debris and provide neurotrophic support for the surviving cells by releasing growth factors.[41] In response to chronic stress in mice, IL-4 signaling-driven hippocampal microglia triggered neurogenesis mediated by brain-derived growth factor.[42] Studies in mouse models of spinal cord injury have shown that exosomes from the M2 subtype of microglia can modulate astrocyte activation and promote axonal regrowth.[43] Microglia can also aid in myelin regeneration, and in part, this reparative microglial response is mediated by infiltering peripheral immune cells.[44]

Peripheral immune cell infiltration during the tertiary phase corresponds with the period of initiation of repair and regeneration. For example, there is a biphasic pattern of myeloid cell recruitment into the neonatal mouse brain after HI, with a second peak 1 week post-HI.[45] Similarly, peripheral adaptive immune cells (T lymphocytes) were recruited into the brain at 1 and 2 weeks, and CD69-expressing B lymphocytes were upregulated in the damaged brain up to 3 months post-HI in neonatal mice.[33] The relative contribution of immune cell to protection and repair after HI in the immature brain needs extensive further research.

Sustained Inflammation in Infants with Neonatal Encephalopathy

Consistent with the preclinical data for persistent inflammation after HI, there is mounting evidence from recent clinical trials that alterations in inflammatory response persist well beyond the early postnatal period in both preterm and term infants with neonatal encephalopathy. Term neonates with HIE treated with therapeutic hypothermia were reported to have higher plasma concentrations of TNF, IL-2, IL-8, and IL-6 at school age than age-matched controls.[46] Prospective cohort studies in term infants with neonatal encephalopathy showed altered cytokine levels in whole blood samples in response to endotoxin exposure during the first 4 days of life, including lower production of pro-inflammatory mediators IL-8, IL-2, IL-6, TNF, granulocyte–macrophage colony-stimulating factor than healthy controls.[47] The difference in baseline and endotoxin-stimulated TNF production was associated with injury severity on MRI. Hypo-responsiveness to stimulation with gram-negative bacterial cell wall mimetics lipopolysaccharide (LPS) was also reported in children with cerebral palsy.[48] In contrast, preterm-born children with periventricular leukomalacia and cerebral palsy were reported to have increased plasma concentration of TNF-α and hyperresponsiveness to inflammatory stimulus at 7 years of age.[49] These findings highlight that the initial response to HI has the potential to either program the immune system for an aggravated response to a secondary inflammatory challenge or induce tolerance to it.

Complex Interaction Between Hypoxia-Ischemia and Inflammatory Insult

Given the high incidence of perinatal inflammation, babies are likely to be exposed to multiple injurious insults. Therefore, there is a need to consider whether pre-existing

inflammation would modify the adaptation to HI and the severity of subsequent neural injury. In a small cohort study in preterm infants, Stark and colleagues showed that histologic chorioamnionitis was associated with increased cerebral oxygen consumption on the first day of life,[50] suggesting that pre-existing inflammation could potentially accelerate metabolic decompensation during HI and alter post-HI recovery. Similarly, in term neonates undergoing therapeutic hypothermia, the presence of histologic chorioamnionitis was associated with worsening of metabolic acidosis within the first 6 hours after birth,[51] implying that the combined insults can exacerbate the severity of HIE.

The additive effect of HI and inflammation is not unexpected. However, considerable preclinical evidence shows that depending on the order, severity, and time interval between the insults, inflammation can modulate the response to HI in a positive (tolerance) or negative (sensitization) manner.[52] For example, in preterm fetal sheep exposed to a chronic low dose of LPS (100–250 ng/day for 5 days), superimposed with bolus injections of 1 μg, there was significantly reduced white matter damage with HI at 4 hours after the last bolus of LPS.[53]

In contrast, sensitization is seen with the shorter or longer intervals between the insults. In P7 rats, exposure to LPS at 2 or 72 hours before HI also exacerbated neural injury.[54] Aggravation of neural injury was associated with altered immune responsiveness to HI, involving TLR4 and the recruitment of the MyD88 adaptor protein and increased NF-kβ signaling.[55] In P9 mice, exposure to LPS 14 hours before HI was associated with a greater acute rise in proinflammatory markers, microglia activation, and neutrophil infiltration compared to HI alone and resulted in progressive exacerbation of diffuse to cystic neural injury over 15 days post-HI.[56]

Sensitization has also been demonstrated in an excitotoxic model of perinatal brain damage. Excitotoxicity is a major mechanism leading to secondary neuronal cell death in neonatal HIE.[18] Intracerebral injection of ibotenate, an agonist of glutamatergic NMDA and metabotropic receptors, at P5 or P10 induces cortical neuronal cell death, mimicking cell death observed in HIE and related animal models.[57,58] Pre-treating the mouse pups (P1-P5) with systemic administration of pro-inflammatory cytokines (IL-1beta, IL-6, or TNF-alpha) significantly exacerbated the excitotoxic neuronal cell death through recruitment of reactive microglia and de-sensitization of glutamate receptors via GRK2 inhibition.[59] Similarly, pre-treatment with a Th2 cytokine (IL-9) in mouse pups exacerbated ibotenate-induced neuronal cell death through mast cell recruitment and histamine release.[60,61]

The increased infiltration of peripheral immune cells also contributes to ongoing neural damage. For example, monocytes infiltrating the brain after inflammation-sensitized HI in neonatal mice transformed into pathologic microglia, which persisted for months after the insult.[62] In rats exposed to a combination of LPS and HI in utero on embryonic day 18, the peripheral blood mononuclear cells were hyper-reactive and had a robust proinflammatory response to LPS stimulation until adulthood.[63] These data suggest a potential increase in long-term vulnerability to subsequent inflammatory challenges. Importantly, altered inflammatory profile could modulate the protective effect of neurotherapeutics.

Efficacy of Therapeutic Hypothermia for Inflammation-Sensitized Hypoxia-Ischemia

Therapeutic hypothermia is now standard care for term neonates with moderate-to-severe HIE in high-income countries. Despite significantly reducing death and disability, therapeutic hypothermia is only partially neuroprotective, with a number needed to treat of 7 ([95% CI 5–10], 8 studies, 1344 infants).[64] Further, a recent large randomized controlled trial has raised concerns that therapeutic hypothermia did not improve outcomes in low- and middle-income settings.[65] The reason why some infants do not

benefit from therapeutic hypothermia is not entirely understood. However, it is postulated that hypothermia treatment might not be effective for neonates with inflammation-sensitized HI injury.[4]

Small studies have reported that in neonates with HIE treated with therapeutic hypothermia, placental abnormalities, including histologic chorioamnionitis, were independently associated with brain injury severity and adverse neurodevelopmental outcomes.[15,66] These findings suggest that therapeutic hypothermia might be less effective in neonates with prior exposure to perinatal inflammation. By contrast, other studies reported no association between placental pathology and MRI findings and neurodevelopmental outcomes after hypothermia treatment.[67,68] Similarly, neonates with sepsis being treated with hypothermia had greater requirements for intensive care support, but they did not have higher mortality.[69] However, the impact on neurodevelopmental outcomes is unknown. Studies with large sample sizes and better-quality evidence are still needed to address the concerns about the effectiveness of therapeutic hypothermia in neonates sensitized with prior infection/inflammation.

Evidence from preclinical studies also supports these concerns. In P7 rats exposed to systemic LPS injection followed by HI after a 4 h delay, LPS sensitization significantly increased brain area loss, apoptosis, microgliosis, and astrogliosis compared with vehicle-HI controls, and treatment with hypothermia did not ameliorate these effects.[70] In contrast, studies in P7 rats using gram-positive bacterial mimetics to induce inflammatory sensitization 8 hours before HI reported that hypothermia was highly neuroprotective,[71] suggesting that hypothermia can still be effective in the presence of gram-positive bacterial infections. Recently, 2 studies have examined hypothermic neuroprotection for LPS-sensitized HI in newborn piglets (human term-equivalent).[72,73] Both these studies reported that hypothermia did not improve acute EEG recovery, MR spectroscopy parameters, and cell survival. These studies used short, sub-optimal durations of therapeutic hypothermia (14–24 hours) and thus there is a need for large animal translational studies using clinical protocols of hypothermia.

Antibiotic Treatment for Perinatal Infections

The efficacy of broad-spectrum antibiotics for managing perinatal infections has been examined for preterm or pre-labor rupture of membranes at term, intra-amniotic infections, and neonatal sepsis.[74] There is no convincing evidence for maternal and neonatal benefit with antibiotic use for prelabor rupture of membranes at term.[75] Indeed, prophylactic antibiotic treatment for preterm rupture of membranes appears to be associated with increased neonatal mortality (Relative Risk 1.57) although there may have been reduced maternal infection.[76] In a randomized control trial in women presenting with spontaneous preterm labor (n = 4221), maternal administration of erythromycin for preterm labor with intact membranes was associated with increased risk of functional impairments and cerebral palsy among the children at 7 years of age.[77] Large randomized control trials are needed to assess the benefits and risks associated with antibiotic regimens for treating early-onset neonatal sepsis. Prolonged use of antibiotics can be associated with antibiotic resistance and longer-term adverse outcomes such as necrotizing enterocolitis and brain injury, especially in preterm neonates without proven infection.[74] Further, in infants undergoing therapeutic hypothermia, the pharmacodynamics of antibiotics may also be modulated by cooling,[78] suggesting the need for careful assessment of dosage and potential adverse effects in infants with perinatal infection and HIE.

Preclinical Studies of Anti-Inflammatory Therapies for Inflammation-Sensitized Hypoxia-Ischemia

In preclinical studies, targeting acute inflammatory processes after inflammation-sensitized HI may confer neuroprotection.[79] For example, in P7 rats exposed to systemic LPS injection and HI after a 4 h delay, intranasally administered of NF-kβ inhibitor (Tat-NBD peptide) at 10 minutes post-insult, reduced atrophy of cortex, striatum, and hippocampus at 1 week post-HI.[55] Similarly, early blockade of peripheral immune cell trafficking into the brain early after LPS–HI using agents such as chemokine receptor antagonist for monocyte blockade, fingolimod for lymphocyte blockade significantly reduces brain tissue loss and improved cognitive function in neonatal rodents.[62,80]

Recent studies have focused on examining the neuroprotective potential of clinically available anti-inflammatory drugs. In P7 rats exposed to gram-negative or gram-positive bacterial mimetics (using LPS or Pam3CysSerLys4) sensitized HI, treatment with 5 doses (22.5 mg/kg, intraperitoneal [i.p.]) of azithromycin starting 2 hours post-HI was associated with improvement in intact tissue volume and sensorimotor function at P35.[81] Similarly, in P4 mice with *Staphylococcus epidermidis*-potentiated HI, i.p. injection of antibiotic vancomycin given 2 minutes after the insult was associated with reduced cortical, deep gray matter and white matter loss at 9 days post-HI.[82]

Recombinant erythropoietin has potent anti-inflammatory properties, and there is preclinical evidence that potentially it could be used to reprogram microglia toward beneficial functions.[83] However, in P17 ferret kits, repeated doses of erythropoietin at 0, 24, 48 hours and 7 days after inflammation-sensitized HI provided no significant neuroprotection.[84] Clinically, high-dose erythropoietin given from 24 hours after birth to 32 weeks postmenstrual age did not improve neurodevelopmental outcomes in extremely preterm infants, suggesting that it is not an effective strategy.[85]

SUMMARY

Inflammation is critical in mediating both secondary and tertiary phase injury and neuro-repair processes after HI. Inflammatory challenges associated with perinatal infections can commonly co-occur with HI. Preclinical studies show exacerbation of neural damage after HI in the presence of pre-existing inflammation and that therapeutic hypothermia may be ineffective for the neural injury associated with a combination of inflammation and HI. However, this needs further investigation in translational large animal studies with optimal duration of hypothermia, as there is no conclusive clinical evidence for the lack or reduced efficacy of neuroprotection with therapeutic hypothermia in neonates with sepsis or chorioamnionitis. Immunomodulatory and anti-inflammatory agents have shown promising neuroprotective effects for inflammation-sensitized HI in neonatal rodents. These studies were limited by the relatively early start time of treatments. Future studies are needed to assess whether these treatments will still be beneficial if started with a clinically realistic delay after the insult and if they will have an additive neuroprotective effect with therapeutic hypothermia.

Best Practices

The current practice for HIE with pre-existing inflammation

- There is considerable speculation in the field that hypothermia treatment might not be effective for neonates sensitized with prior infection/inflammation.

Major recommendations

- Large animal translational studies using clinical protocols of hypothermia and clinical studies with large sample sizes and better-quality evidence are needed to resolve the concerns about the effectiveness of therapeutic hypothermia for inflammation-sensitized HI injury and to test novel add-on therapies.

Bibliographic Source(s)

Mir IN, Johnson-Welch SF, Nelson DB, Brown LS, Rosenfeld CR, Chalak LF. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. Am J Obstet Gynecol. 2015;213(6):849 e1-7.

Wintermark P, Boyd T, Gregas MC, Labrecque M, Hansen A. Placental pathology in asphyxiated newborns meeting the criteria for therapeutic hypothermia. Am J Obstet Gynecol. 2010;203(6):579 e1-9.

Gonzalez FF, Voldal E, Comstock BA, Mayock DE, Goodman AM, Cornet MC, et al. Placental Histologic Abnormalities and 2-Year Outcomes in Neonatal Hypoxic-Ischemic Encephalopathy. Neonatology. 2023;120(6):760-7.

Kovatis KZ, Mackley A, Antunes M, Holmes PJ, Daugherty RJ, Paul D. Relationship Between Placental Weight and Placental Pathology With MRI Findings in Mild to Moderate Hypoxic Ischemic Encephalopathy. Cureus. 2022;14(5):e24854.

Falck M, Osredkar D, Maes E, Flatebo T, Wood TR, Sabir H, et al. Hypothermic Neuronal Rescue from Infection-Sensitised Hypoxic-Ischaemic Brain Injury Is Pathogen Dependent. Developmental neuroscience. 2017;39(1–4):238-47.

Andersen M, Andersen HB, Andelius TCK, Hansen LH, Pinnerup R, Bjerre M, et al. No neuroprotective effect of therapeutic hypothermia following lipopolysaccharide-sensitized hypoxia-ischemia: a newborn piglet study. Frontiers in pediatrics. 2023;11:1,268,237.

Martinello KA, Meehan C, Avdic-Belltheus A, Lingam I, Mutshiya T, Yang Q, et al. Hypothermia is not therapeutic in a neonatal piglet model of inflammation-sensitized hypoxia-ischemia. Pediatr Res. 2022;91(6):1416-27.

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REFERENCES

1. Ahearne CE, Boylan GB, Murray DM. Short and long term prognosis in perinatal asphyxia: an update. World J Clin Pediatr 2016;5(1):67–74.
2. Manuck TA, Rice MM, Bailit JL, et al. Preterm neonatal morbidity and mortality by gestational age: a contemporary cohort. Am J Obstet Gynecol 2016;215(1):103 e1–14.

3. Fox A, Doyle E, Geary M, et al. Placental pathology and neonatal encephalopathy. Int J Gynaecol Obstet 2023;160(1):22–7.
4. Andersen M, Pedersen MV, Andelius TCK, et al. Neurological outcome following newborn encephalopathy with and without perinatal infection: a systematic review. Front Pediatr 2021;9:787804.
5. Abbah J, Vacher CM, Goldstein EZ, et al. Oxidative stress-induced damage to the developing Hippocampus is mediated by GSK3beta. J Neurosci 2022;42(24):4812–27.
6. Kemp MW, Molloy TJ, Usuda H, et al. Outside-in? Acute fetal systemic inflammation in very preterm chronically catheterized sheep fetuses is not driven by cells in the fetal blood. Am J Obstet Gynecol 2016;214(2):281 e1–10.
7. Novak CM, Eke AC, Ozen M, et al. Risk factors for neonatal hypoxic-ischemic encephalopathy in the absence of sentinel events. Am J Perinatol 2019;36(1):27–33.
8. Kim CJ, Romero R, Chaemsaithong P, et al. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol 2015;213(4 Suppl):S29–52.
9. Jain VG, Willis KA, Jobe A, et al. Chorioamnionitis and neonatal outcomes. Pediatr Res 2022;91(2):289–96.
10. Cailes B, Kortsalioudaki C, Buttery J, et al. Epidemiology of UK neonatal infections: the neonIN infection surveillance network. Arch Dis Child Fetal Neonatal Ed 2018;103(6):F547–53.
11. Flannery DD, Edwards EM, Puopolo KM, et al. Early-onset sepsis among very preterm infants. Pediatrics 2021;148(4).
12. Smilga AS, Garfinkle J, Ng P, et al. Neonatal infection in children with cerebral palsy: a registry-based cohort study. Pediatr Neurol 2018;80:77–83.
13. Rao R, Lee KS, Zaniletti I, et al. Antimicrobial therapy utilization in neonates with hypoxic-ischemic encephalopathy (HIE): a report from the Children's Hospital Neonatal Database (CHND). J Perinatol 2020;40(1):70–8.
14. Chalak L, Redline RW, Goodman AM, et al. Acute and chronic placental abnormalities in a multicenter cohort of newborn infants with hypoxic-ischemic encephalopathy. J Pediatr 2021;237:190–6.
15. Mir IN, Johnson-Welch SF, Nelson DB, et al. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. Am J Obstet Gynecol 2015;213(6):849 e1–7.
16. Stone AC, Strickland KC, Tanaka DT, et al. The association of placental pathology and neurodevelopmental outcomes in patients with neonatal encephalopathy. Pediatr Res 2023;94(5):1696–706.
17. Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol 2015;11(4):192–208.
18. Davidson JO, Gonzalez F, Gressens P, et al. Update on mechanisms of the pathophysiology of neonatal encephalopathy. Semin Fetal Neonatal Med 2021;26(5):101267.
19. Fleiss B, Van Steenwinckel J, Bokobza C, et al. Microglia-mediated neurodegeneration in perinatal brain injuries. Biomolecules 2021;11(1).
20. Quan H, Zhang R. Microglia dynamic response and phenotype heterogeneity in neural regeneration following hypoxic-ischemic brain injury. Front Immunol 2023;14:1320271.
21. Paolicelli RC, Sierra A, Stevens B, et al. Microglia states and nomenclature: a field at its crossroads. Neuron 2022;110(21):3458–83.

22. Teo EJ, Chand KK, Miller SM, et al. Early evolution of glial morphology and inflammatory cytokines following hypoxic-ischemic injury in the newborn piglet brain. Sci Rep 2023;13(1):282.
23. Tsuji S, Di Martino E, Mukai T, et al. Aggravated brain injury after neonatal hypoxic ischemia in microglia-depleted mice. J Neuroinflammation 2020;17(1):111.
24. Mallard C, Davidson JO, Tan S, et al. Astrocytes and microglia in acute cerebral injury underlying cerebral palsy associated with preterm birth. Pediatr Res 2014; 75(1–2):234–40.
25. Zhou KQ, Green CR, Bennet L, et al. The role of connexin and pannexin channels in perinatal brain injury and inflammation. Front Physiol 2019;10:141.
26. Davidson JO, Green CR, Bennet L, et al. A key role for connexin hemichannels in spreading ischemic brain injury. Curr Drug Targets 2013;14(1):36–46.
27. Giovannoni F, Quintana FJ. The role of astrocytes in CNS inflammation. Trends Immunol 2020;41(9):805–19.
28. McGowan MM, O'Kane AC, Vezina G, et al. Serial plasma biomarkers of brain injury in infants with neonatal encephalopathy treated with therapeutic hypothermia. Pediatr Res 2021;90(6):1228–34.
29. Mallard C, Ek CJ, Vexler ZS. The myth of the immature barrier systems in the developing brain: role in perinatal brain injury. J Physiol 2018;596(23):5655–64.
30. Mottahedin A, Blondel S, Ek J, et al. N-acetylcysteine inhibits bacterial lipopeptide-mediated neutrophil transmigration through the choroid plexus in the developing brain. Acta neuropathologica communications 2020;8(1):4.
31. Mülling K, Fischer AJ, Siakaeva E, et al. Neutrophil dynamics, plasticity and function in acute neurodegeneration following neonatal hypoxia-ischemia. Brain Behav Immun 2021;92:234–44.
32. Albertsson AM, Zhang X, Vontell R, et al. γδ T cells contribute to injury in the developing brain. Am J Pathol 2018;188(3):757–67.
33. Winerdal M, Winerdal ME, Kinn J, et al. Long lasting local and systemic inflammation after cerebral hypoxic ischemia in newborn mice. PLoS One 2012;7(5): e36422.
34. Herz J, Bendix I, Felderhoff-Muser U. Peripheral immune cells and perinatal brain injury: a double-edged sword? Pediatr Res 2022;91(2):392–403.
35. Parmentier CEJ, de Vries LS, Groenendaal F. Magnetic resonance imaging in (Near-) Term infants with hypoxic-ischemic encephalopathy. Diagnostics 2022;12(3).
36. Geddes R, Vannucci RC, Vannucci SJ. Delayed cerebral atrophy following moderate hypoxia-ischemia in the immature rat. Dev Neurosci 2001;23(3):180–5.
37. Lear BA, Lear CA, Davidson JO, et al. Tertiary cystic white matter injury as a potential phenomenon after hypoxia-ischaemia in preterm f sheep. Brain Commun 2021;3(2):fcab024.
38. Lear CA, Lear BA, Davidson JO, et al. Tumour necrosis factor blockade after asphyxia in foetal sheep ameliorates cystic white matter injury. Brain 2023; 146(4):1453–66.
39. Zhou KQ, Bennet L, Wassink G, et al. Persistent cortical and white matter inflammation after therapeutic hypothermia for ischemia in near-term fetal sheep. J Neuroinflammation 2022;19(1):139.
40. Volpe JJ. Dysmaturation of premature brain: importance, cellular mechanisms, and potential interventions. Pediatr Neurol 2019;95:42–66.
41. Fisch U, Brégère C, Geier F, et al. Neonatal hypoxia-ischemia in rat elicits a region-specific neurotrophic response in SVZ microglia. J Neuroinflammation 2020;17(1):26.

42. Zhang J, Rong P, Zhang L, et al. IL4-driven microglia modulate stress resilience through BDNF-dependent neurogenesis. Sci Adv 2021;7(12).
43. Zhang J, Hu D, Li L, et al. M2 microglia-derived exosomes promote spinal cord injury recovery in mice by alleviating A1 astrocyte activation. Mol Neurobiol 2024. https://doi.org/10.1007/s12035-024-04026-6.
44. Shi L, Sun Z, Su W, et al. Treg cell-derived osteopontin promotes microglia-mediated white matter repair after ischemic stroke. Immunity 2021;54(7):1527–15242 e8.
45. Smith PLP, Mottahedin A, Svedin P, et al. Peripheral myeloid cells contribute to brain injury in male neonatal mice. J Neuroinflammation 2018;15(1):301.
46. Zareen Z, Strickland T, Eneaney VM, et al. Cytokine dysregulation persists in childhood post Neonatal Encephalopathy. BMC Neurol 2020;20(1):115.
47. O'Dea MI, Kelly LA, McKenna E, et al. Altered cytokine endotoxin responses in neonatal encephalopathy predict MRI outcomes. Front Pediat 2021;9:734540.
48. Zareen Z, Strickland T, Fallah L, et al. Cytokine dysregulation in children with cerebral palsy. Dev Med Child Neurol 2021;63(4):407–12.
49. Lin CY, Chang YC, Wang ST, et al. Altered inflammatory responses in preterm children with cerebral palsy. Ann Neurol 2010;68(2):204–12.
50. Stark MJ, Hodyl NA, Belegar VK, et al. Intrauterine inflammation, cerebral oxygen consumption and susceptibility to early brain injury in very preterm newborns. Arch Dis Child Fetal Neonatal Ed 2016;101(2):F137–42.
51. Johnson CT, Burd I, Raghunathan R, et al. Perinatal inflammation/infection and its association with correction of metabolic acidosis in hypoxic-ischemic encephalopathy. J Perinatol 2016;36(6):448–52.
52. Dhillon SK, Lear CA, Galinsky R, et al. The fetus at the tipping point: modifying the outcome of fetal asphyxia. J Physiol 2018;596(23):5571–92.
53. van den Heuij LG, Mathai S, Davidson JO, et al. Synergistic white matter protection with acute-on-chronic endotoxin and subsequent asphyxia in preterm fetal sheep. J Neuroinflammation 2014;11(1):89.
54. Eklind S, Mallard C, Arvidsson P, et al. Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. Pediatr Res 2005;58(1):112–6.
55. Yang D, Sun YY, Lin X, et al. Intranasal delivery of cell-penetrating anti-NF-κB peptides (Tat-NBD) alleviates infection-sensitized hypoxic-ischemic brain injury. Exp Neurol 2013;247:447–55.
56. Bonestroo HJ, Heijnen CJ, Groenendaal F, et al. Development of cerebral gray and white matter injury and cerebral inflammation over time after inflammatory perinatal asphyxia. Dev Neurosci 2015;37(1):78–94.
57. Marret S, Mukendi R, Gadisseux JF, et al. Effect of ibotenate on brain development: an excitotoxic mouse model of microgyria and posthypoxic-like lesions. JNEN (J Neuropathol Exp Neurol) 1995;54(3):358–70.
58. Dommergues MA, Patkai J, Renauld JC, et al. Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. Ann Neurol 2000;47(1):54–63.
59. Degos V, Peineau S, Nijboer C, et al. G protein-coupled receptor kinase 2 and group I metabotropic glutamate receptors mediate inflammation-induced sensitization to excitotoxic neurodegeneration. Ann Neurol 2013;73(5):667–78.
60. Patkai J, Mesples B, Dommergues MA, et al. Deleterious effects of IL-9-activated mast cells and neuroprotection by antihistamine drugs in the developing mouse brain. Pediatr Res 2001;50(2):222–30.

61. Mesplès B, Fontaine RH, Lelièvre V, et al. Neuronal TGF-beta1 mediates IL-9/mast cell interaction and exacerbates excitotoxicity in newborn mice. Neurobiol Dis 2005;18(1):193–205.
62. Chen HR, Chen CW, Kuo YM, et al. Monocytes promote acute neuroinflammation and become pathological microglia in neonatal hypoxic-ischemic brain injury. Theranostics 2022;12(2):512–29.
63. Kitase Y, Chin EM, Ramachandra S, et al. Sustained peripheral immune hyperreactivity (SPIHR): an enduring biomarker of altered inflammatory responses in adult rats after perinatal brain injury. J Neuroinflammation 2021;18(1):242.
64. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev 2013;2013(1):CD003311.
65. Thayyil S, Pant S, Montaldo P, et al. Hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries (HELIX): a randomised controlled trial in India, Sri Lanka, and Bangladesh. Lancet Global Health 2021;9(9):e1273–85.
66. Wintermark P, Boyd T, Gregas MC, Labrecque M, Hansen A. Placental pathology in asphyxiated newborns meeting the criteria for therapeutic hypothermia. Am J Obstet Gynecol 2010;203(6):579 e1–9.
67. Gonzalez FF, Voldal E, Comstock BA, et al. Placental Histologic Abnormalities and 2-Year Outcomes in Neonatal Hypoxic-Ischemic Encephalopathy. Neonatology 2023;120(6):760–77.
68. Kovatis KZ, Mackley A, Antunes M, et al. Relationship between placental weight and placental pathology with MRI findings in mild to moderate hypoxic ischemic encephalopathy. Cureus 2022;14(5):e24854.
69. Sibbin K, Crawford TM, Stark M, et al. Therapeutic hypothermia for neonatal encephalopathy with sepsis: a retrospective cohort study. BMJ Paediat Open 2022;6(1).
70. Osredkar D, Sabir H, Falck M, et al. Hypothermia does not reverse cellular responses caused by lipopolysaccharide in neonatal hypoxic-ischaemic brain injury. Dev Neurosci 2015;37(4–5):390–7.
71. Falck M, Osredkar D, Maes E, et al. Hypothermic neuronal rescue from infection-sensitised hypoxic-ischaemic brain injury is pathogen dependent. Developmental Neuroscience 2017;39(1-4):238–47.
72. Andersen M, Andersen HB, Andelius TCK, et al. No neuroprotective effect of therapeutic hypothermia following lipopolysaccharide-sensitized hypoxia-ischemia: a newborn piglet study. Frontiers in pediatrics 2023;11:1268237.
73. Martinello KA, Meehan C, Avdic-Belltheus A, et al. Hypothermia is not therapeutic in a neonatal piglet model of inflammation-sensitized hypoxia-ischemia. Pediatr Res 2022;91(6):1416–27.
74. Shah NM, Charani E, Ming D, et al. Antimicrobial stewardship and targeted therapies in the changing landscape of maternal sepsis. J Int Med 2024;4(1):46–61.
75. Wojcieszek AM, Stock OM, Flenady V. Antibiotics for prelabour rupture of membranes at or near term. Cochrane Database Syst Rev 2014;2014(10):CD001807.
76. Flenady V, Hawley G, Stock OM, et al. Prophylactic antibiotics for inhibiting preterm labour with intact membranes. Cochrane Database Syst Rev 2013;12:CD000246.
77. Kenyon S, Pike K, Jones DR, et al. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. Lancet 2008;372(9646):1319–27.

78. Matcha S, Raj EA, Mahadevan R, et al. Pharmacometric approach to assist dosage regimen design in neonates undergoing therapeutic hypothermia. Pediatr Res 2022;92(1):249–54.
79. Tetorou K, Sisa C, Iqbal A, et al. Current therapies for neonatal hypoxic-ischaemic and infection-sensitised hypoxic-ischaemic brain damage. Front Synaptic Neurosci 2021;13:709301.
80. Yang D, Sun YY, Bhaumik SK, et al. Blocking lymphocyte trafficking with FTY720 prevents inflammation-sensitized hypoxic-ischemic brain injury in newborns. J Neurosci 2014;34(49):16467–81.
81. Barks JDE, Liu Y, Dopp IA, et al. Azithromycin reduces inflammation-amplified hypoxic-ischemic brain injury in neonatal rats. Pediatr Res 2022;92(2):415–23.
82. Lai JCY, Svedin P, Ek CJ, et al. Vancomycin is protective in a neonatal mouse model of Staphylococcus epidermidis-potentiated hypoxic-ischemic brain injury. Antimicrob Agents Chemother 2020;64(3).
83. Fumagalli M, Lombardi M, Gressens P, et al. How to reprogram microglia toward beneficial functions. Glia 2018;66(12):2531–49.
84. Corry KA, White OR, Shearlock AE, et al. Evaluating neuroprotective effects of uridine, erythropoietin, and therapeutic hypothermia in a ferret model of inflammation-sensitized hypoxic-ischemic encephalopathy. Int J Mol Sci 2021;22(18).
85. Juul SE, Comstock BA, Wadhawan R, et al. A randomized trial of erythropoietin for neuroprotection in preterm infants. N Engl J Med 2020;382(3):233–43.