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Therapeutic Hypothermia in Neonatal Hypoxic-Ischemic Encephalopathy.

Guido Wassink, Joanne O. Davidson, Simerdeep K Dhillon, Kelly Q Zhou ...+4 more authors

Institutions: University of Auckland, University of Oslo, University of Bristol

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Therapeutic hypothermia in neonatal hypoxic-ischemic encephalopathy

Guido Wassink, PhD,[1] Joanne O. Davidson, PhD,[1] Simerdeep K. Dhillon, BSc,[1] Kelly Zhou, BSc[1] Laura Bennet, PhD,[1] Marianne Thoresen, MBChB, PhD,[2] Alistair J. Gunn, MBChB, PhD.[1]

1. Dept of Physiology, School of Medical Sciences, University of Auckland

2. Translational Health Sciences, University of Bristol, UK and Division of Physiology, University of Oslo

Correspondence to:
Alistair Jan Gunn,
Dept of Physiology,
Faculty of Medical and Health Sciences
The University of Auckland
Private Bag 92019, Auckland, New Zealand
Phone number: [64](9) 3737599 ext. 96763
E-mail: aj.gunn@auckland.ac.nz

ORCID: 0000-0003-0656-7035

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Abstract

Purpose of review - therapeutic hypothermia reduces death or disability in term and near-term infants with moderate-severe hypoxic-ischemic encephalopathy. Nevertheless, many infants still survive with disability, despite hypothermia, supporting further research into ways to further improve neurologic outcomes. **Recent findings** - recent clinical and experimental studies have refined our understanding of the key parameters for hypothermic neuroprotection, including timing of initiation, depth, and duration of hypothermia, and subsequent rewarming rate. However, important knowledge gaps remain. There is encouraging clinical evidence from a small phase II trial that combined treatment of hypothermia with recombinant erythropoietin further reduces risk of disability but definitive studies are still needed. **Summary -** In conclusion, recent studies suggest that current protocols for therapeutic hypothermia are near-optimal, and that the key to better neurodevelopmental outcomes is earlier diagnosis and initiation of hypothermia after birth. Further research is essential to find and evaluate ways to further improve outcomes after hypoxic-ischemic encephalopathy, including add-on therapies for therapeutic hypothermia and preventing pyrexia during labor and delivery.

Introduction

Hypoxic-ischemic encephalopathy (HIE) around the time of birth represents the single greatest contribution to overall disability worldwide and accounts for one-tenth of all disability-adjusted life years [1]. Even in the developed world, HIE occurs in approximately 2 to 3/1000 live births at term and near-term [2]. HIE is initiated before labor in only about 10% of cases as shown by chronic fetal heart rate changes [3], and studies using magnetic resonance imaging (MRI) confirm that the great majority of infants with acute HIE do not have established brain atrophy [4], suggesting that most brain injury occurs around the time of birth.

The major advance in neonatal care of HIE has been the successful translation of therapeutic hypothermia in to routine practice [5]. Compelling clinical evidence from multiple randomized trials shows that therapeutic hypothermia improves survival without disability to infancy and mid-childhood, and, moreover, significantly reduces the most severe disability, cerebral palsy [6]. Nevertheless, a substantial proportion of infants still survive with disability despite treatment with hypothermia. At the time that the large randomized trials were carried out, many aspects of the treatment protocols were based on best estimates. The key aim of this review is to examine recent studies that have helped refine our understanding of the critical parameters for neuroprotection with therapeutic hypothermia, and to highlight knowledge gaps.

Cerebral damage after hypoxia-ischemia: an 'evolving' process

The seminal observation that enabled the translation of therapeutic hypothermia to clinical practice was that brain cell death is not necessarily limited to the period of hypoxia-ischemia (HI, the 'primary' phase of injury), but that HI can precipitate a cascade of biochemical processes that lead to delayed cell death hours or even days afterwards. It is now well established in term infants and animals that even after severe HI there is often considerable cell survival with recovery of oxidative mitochondrial metabolism in a 'latent' phase characterized by suppression of neural activity and hypoperfusion [7, 8], followed by progressive secondary failure of energetic cellular metabolism [9], with late onset of stereotypic seizures, cytotoxic edema (i.e. pathological cell swelling, Figure 1) [7] and

extracellular accumulation of excitatory amino acids due to reuptake failure by astroglia and excessive depolarization-mediated release [8]. The onset of energetic failure after hypoxia-ischemia is tightly coupled with the appearance of histologic brain damage in animal studies [10], and, in human infants, correlates with neurodevelopmental outcome at 1 and 4 years of age [11]. More severe HI is typically associated with greater primary cerebral damage [12], earlier onset of secondary deterioration, greater neuronal loss [13, 14], and increased risk of mortality and adverse neurological outcomes in newborns with HIE [11]. Experimentally, the secondary energetic metabolic failure is associated with irreversible release of cytochrome c, the terminal electron acceptor of oxidative cellular respiration, from the mitochondria [15], indicating that it is mainly a function of evolving cell death. Hence, it is during this relatively short 'latent' phase before secondary cellular deterioration that therapeutic interventions are most likely to improve outcomes.

This concept, that acute, global hypoxia-ischemia can trigger evolving brain damage with characteristic events appearing at different times after the insult, is central to understanding studies of neuroprotection.

Therapeutic hypothermia – from bench to cotside

Compelling preclinical evidence for hypothermic neuroprotection *after* hypoxia-ischemia, from multiple paradigms [16], led to trials of induced mild hypothermia for moderate to severe neonatal HIE. Systematic meta-analysis of 11 randomized controlled clinical trials (RCTs) of selective head cooling and whole-body cooling initiated within 6 h of birth, involving 1505 term and near-term infants (>=35 weeks gestation) with moderate to severe HIE found consistent beneficial effects after hypothermia [17]. In these trials, mild induced hypothermia was associated with reduced risk of death or major neurodevelopmental disability by 18 months of age (relative risk (RR) 0.75 (95% confidence interval (CI) 0.68 to 0.83). Importantly, cooling reduced mortality (RR 0.75 (95% CI 0.64 to 0.88), 11 studies, 1468 infants), and reduced neurodevelopmental disability in survivors (RR 0.77 (95% CI 0.63 to 0.94), 8 studies, 917 infants).

Long-term follow-up of these studies is still ongoing; the available evidence suggests a similar improvement in outcomes in middle childhood after mild induced hypothermia for HIE [18-

20]. For example, the Total Body Hypothermia for Neonatal Encephalopathy (TOBY) Trial found that more children in the hypothermia group survived without neurologic abnormalities than in the control group (45% vs. 28%; RR 1.60, 95% CI: 1.15–2.22) [19], with an IQ score of 85 or higher (52% vs. 39%; RR 1.31, P=0.04). Consistent with these findings at 18 months of age, there was a reduced risk of cerebral palsy (21% vs. 36%, P=0.03) and moderate or severe disability (22% vs. 37%, P=0.03).

Which is better - head or whole-body cooling?

The brain can be cooled through whole-body or selective head cooling. In essence, systemic cooling provides homogenous hypothermia to the cerebral cortex and deep brain structures, whereas selective head cooling cools the cortex more than the central brain regions [21]. Given that hypoxic-ischemic brain damage often involves cortex and subcortical structures (i.e. basal ganglia and thalamus), either strategy is reasonable in principle. There is limited evidence for superiority of one compared to the other. For example, a small cohort study found a significant reduction in severe cortical lesions with head cooling, but not with body cooling, whereas subcortical lesions were equally reduced in both cooling groups in neonates with milder aEEG abnormalities [22]. By contrast, another small cohort of infants suggested a higher incidence of severe mixed lesions after head cooling compared to whole body cooling [23]

In practice, comprehensive meta-analysis of 7 cooling trials showed highly similar reductions in neonatal mortality or major disability with head and whole-body hypothermia in infants with HIE [24], and so these strategies appear equivalent. However, it is easier to implement systemic cooling with servo-controlled temperature regulation, and thus whole body cooling is now used more widely.

Is it possible to optimize current protocols for therapeutic hypothermia?

Current protocols for therapeutic hypothermia are incompletely neuroprotective, with a number needed to treat of 7 to 9 ((95% CI 5 to 10), 8 studies, 1344 infants) [17]. As reviewed, the experimental efficacy of hypothermia is highly dependent on *timing of initiation*, and *depth* and *duration* of cooling [25]; critical parameters that were in part based on pilot studies and

empiric decisions. This suggested the possibility that it is possible to further optimize the clinical regimens for therapeutic hypothermia.

At the same time, it is important to appreciate that there is some evidence that outcomes may have improved now that therapeutic hypothermia is routine care for moderate to severe HIE. In a recent randomized controlled trial of cooling strategies, the rate of death or disability at 18 months of age in infants treated with cooling to 33.5°C for 72 h was 29.3% [26], compared with 44% in infants receiving the same cooling protocol in a previous trial that used the same recruitment criteria [27]. Consistent with this, recent non-randomized cohort studies of infants cooled for HIE suggest both reduced severity of cerebral palsy [28] and lower incidence of epilepsy at 2 years of age compared with the original cooling trials [29]. The factors behind these apparent improvements in outcome after therapeutic hypothermia are not known. Speculatively, they might be related to recruiting infants with less severe HIE as shown by less severe Apgar scores in the recent trial [30]. Alternatively, it is plausible that cooling is being initiated earlier, with greater use of very early passive cooling while infants with moderate-severe HIE are assessed for active cooling [31].

The timing of starting hypothermia - more neuroprotection with less delay

Delaying initiation of cooling dramatically reduces its efficacy in animal studies [14]. Studies in infant and adult rodents and in near-term fetal sheep showed that neuroprotection for cooling started within 6 hours after HI could be achieved by extending the duration of hypothermia to 48 to 72 hours, until secondary phase events such as high amplitude seizures and cytotoxic edema resolved [25]. This concept is highly consistent with the delayed onset of mitochondrial failure observed during the latent phase on MRI after moderate to severe HIE in human infants [9, 11] and piglets [32] and on near infrared spectroscopy in fetal sheep. The effect of delayed initiation was difficult to analyse in the RCTs of therapeutic hypothermia because the great majority of infants were randomized late in the latent phase. However, in support, a subsequent cohort study of 65 surviving cooled newborns showed that 43 infants in whom cooling was started before 3 hours of age had significantly better Psychomotor Development Index (PDI) scores (median PDI: 90) than those who were cooled after 3 hours (median PDI: 78) [30].

How deep is optimal for hypothermic neuroprotection?

Similarly, the critical depth of hypothermia required for protection may be affected by multiple factors including the delay before initiation and the severity and nature of the insult. There is some evidence from studies in adult rodents and in fetal sheep that when cooling was delayed until 6 hours after cerebral ischemia greater functional and histological neuroprotection was achieved with a 5°C reduction than with 3°C [33, 7]. However, in 7-day old rat pups, cooling to 32, 30, 26, or 18°C for 5 h after hypoxia-ischemia, was not associated with additional neuroprotection compared to 33.5°C [34]. Similarly, in neonatal piglets, whole-body hypothermia after cerebral ischemia with a reduction in body temperature of either 3.5 or 5°C was associated with significant and highly similar neuroprotection, whereas a reduction of 8°C was detrimental [35]. Finally, a large randomized controlled clinical trial has confirmed that cooling infants with moderate to severe HIE to 32°C instead of 33.5°C did not further reduce death or moderate to severe disability at 18 months of age [26]. These consistent clinical and pre-clinical findings suggest that there is a relatively broad range of cooling temperatures beneficial for the brain after hypoxia-ischemia, and that, reassuringly, it should not be necessary to reduce core temperatures by more than ~3.5°C.

If some cooling is good, would more be even better?

The original RCT protocols required that hypothermia should be continued for 72 hours, based on evidence from animal studies that cooling needed to be continued for between 48 and 72 hours [36, 7]. However, it is not clear whether shorter or more prolonged durations of cooling might be either sufficient or potentially more effective, respectively. Robust recent preclinical studies now suggest that continuing hypothermia for 72 hours is needed for optimal neuroprotection [37, 38]. For example, in near-term fetal sheep, delayed cerebral cooling, started 3 h after cerebral ischemia and continued until 48 hours, provided partial protection but was substantially less effective for recovery of EEG power and neural survival than cooling for 72 hours (Figure 1) [38]. Conversely, in the term-equivalent fetal sheep, when delayed hypothermia started 3 hours after ischemia was prolonged from 3 to 5 days, there was no further improvement in electrophysiological recovery or neuronal survival or reduction in

cortical microglial induction [37]. Indeed, extended cooling was associated with a small reduction in neuronal survival in the parasagittal cortex and dentate gyrus.

These preclinical findings have been confirmed by a large clinical trial in 364 infants with moderate to severe HIE of prolonged duration and increased depth of therapeutic hypothermia. This study was stopped at 50% of planned recruitment due to lack of effect and safety concerns [39, 26]. The adjusted risk ratio for death in the neonatal intensive care unit after cooling for 120 hours compared to 72 hours was 1.37 (95% CI: 0.92–2.04) and for the 32°C compared to 33.5°C was 1.24 (CI: 0.69–2.25). There was no significant overall effect of longer or deeper cooling on death or disability at a mean age of 18 months [26].

Is speed of rewarming after therapeutic hypothermia important?

The optimal rate of rewarming after therapeutic hypothermia for HIE is unknown, albeit it is widely suggested that slow rewarming is beneficial. The randomized clinical trials of therapeutic hypothermia for HIE recommended rewarming neonates at no more than 0.5°C per hour [40]. This was largely based on case reports that fast rewarming might destabilize cardiovascular function [41], or trigger rebound seizures [42]. However, there is no controlled human evidence for the optimal rate of rewarming.

There is limited evidence from animal studies that rapid rewarming may reverse the depression of potential injurious processes such as oxidative stress or excitotoxin release [43, 44], and slow rewarming may improve neural outcomes. In neonatal piglets exposed to severe HI, hypothermia for 18 hours followed by rewarming at 0.5°C/h was associated with less caspase-3 activation in the cerebral cortex and white matter tracts than with rewarming at 4°C/h [45, 46]. Moreover, in adult gerbils subjected to transient forebrain ischemia, fast rewarming after hypothermia for 2 hours was associated with transient uncoupling of cerebral blood flow and metabolism and loss of neuroprotection in the CA1 region of the hippocampus, which was prevented by slow or stepwise rewarming [47]. However, these studies tested very short intervals of cooling that are likely to have been highly sub-optimal.

In near-term fetal sheep, cerebral hypothermia started 3 hours after global cerebral ischemia and continued for 48 hours, was associated with a striking deterioration in EEG power and

significantly less neuroprotection than 72 hours of hypothermia [38]. In that study, fetuses were allowed to rewarm spontaneously, typically reaching control temperatures in approximately one hour [48]. After 48 hours of cerebral hypothermia very slow rewarming over 24 hours (~0.2°C/h) was associated with improved electrographic recovery compared with rapid rewarming (~5°C/h). However, neuronal survival in the cortex and CA4 was still significantly less than after 72 hours of hypothermia with fast rewarming ($p<0.05$) [49]. Further studies are needed to test typical clinical rewarming regimes after 72 hours of hypothermia, but these data strongly suggest that the overall duration of cooling was more important than speed of rewarming after therapeutic hypothermia.

Is there benefit from cooling initiated later than 6 hours after birth?

The preclinical and clinical studies reviewed above consistently suggest that hypothermia should be started as early as possible in the first 6 hours of life to achieve optimal outcomes. However, some infants are unable to be started within this time window, because of late diagnosis or being outborn in areas that cannot provide support for cooling. Even though it is not optimal, should these infants be offered therapeutic hypothermia after 6 hours of life? A recent randomized controlled trial conducted by 21 Neonatal Research Network centers in the USA recruited 168 term infants with HIE, in whom therapeutic hypothermia could not be started within 6 hours of life. Infants were randomized between > 6 and <24 hours after birth to either hypothermia (33 to 34°C) or normothermia (36.5 °C to 37.3°C). The median time to start cooling in this trial was 16 hours after birth. Neurodevelopmental outcome was assessed in survivors at 18-22 months with the Bayley III. Outcome was available for 78 cooled (9 died and 10 had moderate to severe disability) and 79 normothermic patients (9 died and 13 had moderate to severe disability) [50]. This difference was not significant ($p >0.5$, Chi-squared test). A Bayesian analysis suggested a 71, 64 or 56% probability of reducing death or disability by at least 1, 2 or 3%, respectively. Taken as a whole, this study strongly reinforces the need for strong clinical protocols to ensure that therapeutic hypothermia is started in the first 6 hours after resuscitation for the best chance of a favorable outcome.

Can we improve care during labor and resuscitation?

Collectively, these studies infer that the most likely improvement in outcomes from therapeutic hypothermia would result from starting treatment as early as possible within the first 6 hours after birth. This strongly suggests that a key aspect of neonatal management must be to avoid pyrexia during labor and resuscitation.

Fetal temperatures are higher than maternal temperatures, as the mother acts as a heat sink for the fetus. Thus, maternal pyrexia increases fetal temperature and risk of neural injury during HI. In pregnant baboons raising the maternal temperature to between 41 and 42 ºC increased uterine activity and the temperature gradient between fetus and mother from 0.47 ºC to 0.75 [51], and was associated with severe fetal acidosis and hypoxia, hypotension and tachycardia. Although there are no randomized controlled trials of temperature control in labor, fever during labor has been independently associated in multiple studies with neonatal morbidity, including death, neonatal seizures, encephalopathy and neonatal stroke [52-55].

Thus, studies of controlling pyrexia in labor are highly desirable and should be developed urgently. Pragmatically, it is now widely considered that overhead heaters should be turned off during resuscitation of term or near-term infants. During subsequent observation, if encephalopathy is suspected, the infants should be managed first with mild passive hypothermia followed with active cooling, with strict temperature monitoring. In addition to controlling for pyrexia, therapeutic hypothermia is started much sooner after birth using this approach. For example, neonates cooled passively during transport reached their target temperature (33.5ºC) approximately 2 h faster than infants that were not cooled until arrival at the referral center [56]. In a small cohort of 50 infants that received hypothermia, time from birth to achieve core temperature <34 °C was 2.6 ± 1.8 h for inborn infants, 3.9 ± 1.6 h for infants cooled before and during transport, and 9.8 ± 6.2 h for outborn infants who were not cooled until arrival at the hypothermia center [57]. Whether this translates into improved hypothermic neuroprotection is still unknown.

Other populations: Should we cool infants with mild HIE?

The large RCTs of therapeutic hypothermia excluded infants who had mild HIE in the first 6 hours of life as it was unknown whether they were at risk of adverse outcomes, and so the potential benefit of treating these infants with therapeutic hypothermia is unknown. There is

now evidence from cohort studies that some infants with mild HIE, as defined using the trial criteria, in the first 6 hours of life have a significant risk of disability [58]. The evidence is rather variable, likely because of variable criteria for mild HIE, retrospective identification, less formal neurological examinations than used in the prospective trials, or not using aEEG criteria. In practice, some of these infants would very likely have been classified as having evolved to Stage II (moderate) encephalopathy by Sarnat and Sarnat by 24 h [59], because the historical criteria required longitudinal assessment of neurological progress until hospital discharge or death plus multimodal assessment, typically including a formal EEG and imaging, whereas, in the modern setting, severity is assessed by clinical criteria only within the first 6 hours of life. Thus, given the evolving nature of HIE, it is not really possible to accurately define mild HIE in the first 6 hours of life.

One meta-analysis that included studies with well-defined HIE grading at birth, and standardized neurodevelopmental assessment at 18 months or older, suggested that 86/341 (25%) of infants with mild HIE in the first 6 hours of life had an adverse outcome [60], defined as death, cerebral palsy or neurodevelopmental test scores that were more than 1 standard deviation below the mean. A very recent prospective cohort study of mild HIE defined as ≥1 neurological abnormality using modified Sarnat criteria within 6 h of birth and not meeting cooling criteria, found that 16% of infants had disability at a mean of 19 months [61]. Indeed, 40% of infants had Bayley scores more than one standard deviation below the mean (< 85) for either cognition, motor, or language. Similarly, a prospective cohort study of infants who were not treated with therapeutic hypothermia, suggested that infants with mild HIE, determined by both early EEG and clinical examination, had adverse cognitive and neuromotor outcomes at 5 years of age [62]. Interestingly, although intact survival was much greater after mild than moderate or severe HIE, survivors showed no significant difference in cognitive outcomes between those who had had mild compared to moderate HIE.

Given that this population of infants with mild HIE in the first 6 hours is heterogeneous, the balance of clinical risk and benefit is unclear. Treating all such cases would increase the numbers of infants being separated from their parents, receiving invasive treatments such as central lines, invasive respiratory support, sedation and delayed oral feeding. There is no established prognostic method by which clinicians can determine if it is possible to stop

cooling before 72 hours [63], and so hypothermia should be continued for 3 days. Supporting this concept, in one case series of 10 infants with mild HIE in whom hypothermia was stopped early because of rapid clinical improvement, neural injury on magnetic resonance imaging was seen in half and disability at 2 years of age in 2 infants [64]. Given that there are roughly as many infants with mild HIE as there are with moderate to severe HIE, it is critical that the benefits of treatment for this group should be formally tested.

Summary

In summary, current evidence suggests that current protocols of whole body or head cooling for 72 hours are reasonably close to optimal. Thus, the most effective way to further improve outcomes from therapeutic hypothermia in infants with HIE is to initiate cooling earlier, as soon as possible in the first 6 hours after birth. Alternatively, hypothermia combined with other pharmacological or non-pharmacological interventions may enable further improvements in outcome. There is currently considerable interest in such options, based on the endogenous induction of potentially protective compounds in the brain as well as exogenous agents [65].

What is the potential for add-on therapies?

There is strong evidence that both programmed cell death and sterile post-ischemic inflammation augment cell death pathways in the latent phase, and some evidence that oxygen free radicals and excitotoxins may also play a role [65]. Thus, it is reasonable to consider whether agents modulating these pathways might augment therapeutic hypothermia. There are many trials in progress, as listed in Table 1. The results of recent clinical trials have been mixed. For example, in a phase-II trial in newborns with HIE treated with hypothermia, treatment with an anticonvulsant, topiramate (n=21), was safe, but did not improve death or neurological disability compared with cooling alone (n=23) [66]. The noble gas Xenon has significant anti-apoptotic effects via the N-methyl-D-aspartate (NMDA) receptor in piglets [67], but did not augment hypothermic neuroprotection in a small phase-II trial (n=46 for both groups) as assessed by magnetic resonance spectroscopy measures, including lactate to N-acetyl aspartate ratio in the thalamus (mean ratio: 1.09; 95% CI: 0.90-1.32) and fractional anisotropy (mean difference: -0.01; 95% CI: 0.03-0.02) in the posterior limb of the internal capsule [68]. However, this study was limited by delay in starting Xenon until ~10 h after

birth, which is outside its reported therapeutic window. A second RCT is still in progress (Table 1). More encouragingly, a small trial of 30 infants with HIE randomized to receive either the endogenous hormone melatonin plus hypothermia or hypothermia alone, reported improved survival at 6 months of age without neurologic or developmental abnormalities [69]. However, these findings are preliminary and must be validated in larger trials.

One of the more promising neurotherapeutics, recombinant erythropoietin (rEpo), has anti-apoptotic, anti-oxidant, anti-excitotoxic and anti-inflammation effects in preclinical paradigms of neonatal brain damage [25]. In addition, it promotes proliferation as well as maturation and differentiation of oligodendrocytes and neurons, which at least in principle could help promote neurorepair after HI. Small randomized trials in term infants with HIE have reported improved outcomes on modern imaging and neurologic measures after monotherapy with rEpo [70-72]. Further, a phase-II double-blinded, placebo-controlled randomized trial in infants with moderate to severe HIE who received hypothermia plus multiple rEpo doses (1000 U/kg, i.v. at 1, 2, 3, 5, and 7 days post-birth), compared to cooling plus placebo, showed reduced subcortical and cerebellar brain damage scores on MRI at a mean of 5.1 days, with better motor and developmental scores at 12.7 months of age [73]. Two large phase III trials of hypothermia plus rEpo are currently recruiting (Table 1).

CONCLUSION

Therapeutic hypothermia is now established as standard care to improve neurological recovery in infants with moderate to severe hypoxic-ischemic encephalopathy. However, current protocols are only partially effective [17]. Further improvement in neurodevelopmental outcomes is likely to come from strategies to avoid pyrexia during and after resuscitation, to start hypothermia earlier, and co-treatment with endogenous or exogenous neuroprotective agents. Early EEG recordings and other biomarkers can help to identify patients who would benefit from treatment in such a limited time frame [74]. Endogenous neuroprotective compounds such as rEpo are showing particular promise, with multiple potential benefits and excellent safety records in other settings.

The key priorities for further research include:

1. Large pragmatic trials to test therapeutic hypothermia in new populations, in particular, evaluating the risks and benefits of therapeutic hypothermia for infants with mild HIE.

2. Studies of improved care in labor, particularly trials to test whether preventing maternal pyrexia can reduce the risk of neonatal hypoxic-ischemic encephalopathy.

3. Preclinical studies to identify and develop more effective neuroprotective interventions both in combination with therapeutic hypothermia and for use in settings where hypothermia is inappropriate, such as in extremely preterm infants.

4. Identifying and refining biomarkers such as EEG recordings to robustly identify infants with encephalopathy who would benefit from treatment as soon as possible after birth.

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Table 1. Active trials of neurotherapeutics and therapeutic hypothermia in term neonates with hypoxic-ischemic encephalopathy.

Registration	Clinical Trial	Phase	Treatment Regimen	Outcome Measures	Principal Investigator
01. NCT02621944	Melatonin as a Neuroprotective Therapy in Neonates with HIE Undergoing Hypothermia	I	0.5-5.0 mg/kg Melatonin, enteral doses starting <12 h of birth, combined with therapeutic hypothermia*	MRI at 7-12 days of age, BSID-III Index Score at 18-20 months, and GMFA at 3 and 23 months of age	University of Florida, USA (Michael D Weiss, MD)
02. NCT02812433	Sildenafil Administration to Treat Neonatal Encephalopathy (SANE Trial)	I	2 mg/kg/dose Sildenafil per os, twice daily on day 2-9 after birth, combined with therapeutic hypothermia	Adverse events at day 1-14, MRI at 2 and 30 days, BSID-III Index Scores at 1 and 2 years of age	McGill University, Canada (Pia Wintermark, MD)
03. NCT02700854	Hypoxic-Ischemic Encephalopathy Therapy Optimization in Neonates for Improved Protection With CO2 (HENRIC Trial)	I	5% CO_2 through patient circuits in ventilators, started <6 hours of birth for 12 h max, with therapeutic cooling	Death, number seizures <1 week, Lac/NAA ratio on MRS, fractional anisotropy on MRI, hypotension	Semmelweis University, Hungary (Miklos Szabo)
04. NCT01765218	Topiramate in Neonates Receiving Whole Body Cooling for Hypoxic-Ischemic Encephalopathy	I/II	5.0 mg/kg Topiramate, enteral doses, given daily for 5 days from admission, combined with therapeutic cooling	Seizures <4 weeks or discharge, MRI at day 5-7, BSID-III Scores at 9, 18 and 27 months of age	University of California, USA (Kristin Hoffman, MD)
05. NCT02551003	Protective Effect of Autologous Cord Blood combined with Therapeutic Hypothermia Following Neonatal Encephalopathy	I/II	Autologous Cord Blood, i.v. <24 hours, and at 48 and 72 h of life, combined with therapeutic hypothermia	MRI at 7 and 28 days and 12 months, death or disability; BSID-III Score at 18 months of age	Fudan University (Wenhao Zhou, MD)
06. NCT02881970	Neonatal HIE : Safety and Feasibility Study of a Curative Treatment With Autologous Cord Blood Stem Cells	I/II	Autologous cord blood stem cells; unknown regimen, combined with therapeutic hypothermia	Adverse clinical or paraclinical events, and neurodevelopmental	Hopitaux de Marseille (Catherine Geindre, PhD)

	(NEOSTEM Trial)			outcomes at 22-24 months	
07. NCT02071394	Xenon and Cooling Therapy in Babies at High Risk of Brain Injury Following Poor Condition after Birth (CoolXenon3 Trial)	II	50% Xenon through patient circuits in ventilators, started <5 h of birth for 18 hours, with therapeutic hypothermia	MRI <2 weeks of life, death or moderate to severe impairment; BSID-III Score at 18-24 months	University of Bristol, UK (Marianne Thoresen, PhD)
08. NCT02434965	Autologous Cord Blood and Human Placental-Derived Stem Cells in Neonates With Hypoxic-Ischemic Encephalopathy (HPDSC+HIE Trial)	II	Autologous Cord Blood and HPDSC, i.v. unknown dose <24 hours and on days 2, 3, 7 and 8 following birth	Adverse clinical events <30 days, neurologic condition; MRI, DTI and Sarnat exam <24 months	New York Medical College, USA (Mitchell Cairo, MD)
09. NCT02612155	A Multi-Site Study of Autologous Cord Blood Cells for Hypoxic-Ischemic Encephalopathy	II	Autologous Cord Blood, i.v. 2 infusions, started <48 hours of life, combined with therapeutic hypothermia	Seizures, death or moderate to severe impairment; BSID-III Index Scores at 12 month of age	Duke University, USA (Michael Cotten, MD)
10. NCT03071861	Mild Encephalopathy in the Newborn Treated With Darbepoetin-Alpha (MEND Trial)	II	10 mcg/kg Darbepoetin Alpha i.v. single dose, given <24 h after birth	Adverse events or seizures <30 days, neurologic outcome; GMFA and BSID-III at 12-18 months	University of New Mexico, USA (John Philips, MD)
11. NCT03409770	Optimising the Duration of Cooling in Mild Encephalopathy (COMET Trial)	II/III	Whole-body hypothermia (33-34°) started <6 h for 24, 48 or 72 hours, with rewarming at 0.5°C per hour	MRI and thalamic N-acetyl aspartate ratio on MRS, obtained at 4 to 14 days following birth	Imperial College London, UK (Pete Lally, PhD)
12. NCT03162653	Effect of Allopurinol for Hypoxic-Ischemic Brain Injury on Cognitive Outcome (ALBINO Trial)	III	20 mg/kg Allopurinol, first i.v. dose <30 min of birth, 10 mg/kg second dose at 12 hours, combined with hypothermia	Death or neurodevelopmental impairment; GMFA and BSID-III Index Scores at 24 months of age	University Hospital Tuebingen, Germany (Rudiger Mario, PhD)
13.	High-dose Erythropoietin for	III	1000 IU/kg rEpo i.v. <24	Death or moderate to	University of

NTC02811263	Asphyxia and Encephalopathy (HEAL Trial)		h, and at 2, 3, 4, and 7 days after birth, combined with therapeutic hypothermia	severe impairment; GMFA and BSID-III Scores at 22-26 months of age	California, USA (Yvonne Wu, MD)
14. NCT03163589	Erythropoietin in Management of Neonatal Hypoxic-Ischemic Encephalopathy	III	1000 IU/kg rEpo i.v. <24 h, and at 2, 3, 5, 7 and 9 days after birth, combined with therapeutic hypothermia	MRI <2-3 weeks of life, death or moderate-severe impairment; Griffith Score at 12 months of age	Assiut University, Egypt (Samia Mohamed, MD)
15. NCT03079167	Erythropoietin for Hypoxic-Ischemic Encephalopathy in Newborns (PAEAN Trial)	III	1000 IU/kg rEpo i.v. <23 h, and at 2, 3, 5, and 7 days after birth, combined with therapeutic hypothermia	Death or moderate to severe impairment; GMFA and BSID-III Index Scores at 24 months of age	University of Sydney, Australia (Helen Liley, MD)

All ongoing single and multi-center trials investigating the effects of neurotherapies and therapeutic hypothermia in neonates with hypoxic-ischemic encephalopathy. Trials registered at ClinicalTrials.gov. HIE, hypoxic-ischemic encephalopathy; MRI, magnetic resonance imaging; BSID, Bayley Scale of Infant Development; GMFA, Generalized Motor Function Assessment; per os, per mouth; CO_2, carbon dioxide; Lac/NAA ratio, lactate/N-acetyl-aspartate ratio; MRS, magnetic resonance spectroscopy; IU, international units; rEpo, recombinant human erythropoietin; i.v., intravenous; HPDSC, human placental-derived stem cells; DTI, Diffusion Tensor Imaging.

Figure Legend

Figure 1. Changes in fetal extradural temperature (top panel), cortical impedance (middle panel) and electroencephalogram (EEG) power (bottom panel), after 30 minutes of global cerebral ischemia in the near-term fetal sheep. Cortical impedance is a measure of cellular swelling in the parietal cortex. Fetuses received either sham-ischemia plus sham-cooling (clear), ischemia with sham cooling (black) or cerebral hypothermia started 3 hours after reperfusion, and continued until 48 hours (red), 72 hours (blue), or 120 hours (green). Cerebral ischemia starts at time zero. All hypothermia groups showed complete suppression of secondary rise in cortical impedance (i.e. it abolished delayed cell swelling), and substantially greater recovery of EEG power after resolution of late seizures. However, hypothermia for 48 hours was associated with relapse of EEG power when cooling was discontinued, and reduced cortical impedance from 120 hours onwards (i.e. which is believed to represent cell loss). There was no significant difference between hypothermia for 3 days or 5 days in either EEG power or cortical impedance. Histological analyses showed loss of neuronal cells in the parasagittal cortex in the ischemia sham-cooling, and hypothermia for 2 and 5 days groups, compared to sham-ischemia sham-cooling. Neuronal survival in the cortex was greatest in the ischemia 3-day hypothermia group (see insert). Data are mean ± SEM. *P<0.05 vs. sham-ischemia sham-cooling group, #P<0.05 vs. ischemia-sham cooling group. Data derived from Davidson et al [37, 38].