

Delayed Neuroprotection in the Era of Hypothermia : What Can We Add?

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Abstract

Despite the successful clinical translation of therapeutic hypothermia for perinatal encephalopathy into routine care, treatment is only partially effective. It is likely that this reflects the formidable challenges of initiating treatment for neonatal encephalopathy within a few hours after birth.

In randomized controlled trials, cooling has been typically initiated at a mean of 4 to 5 h after birth. This is clearly not optimal, given accumulating evidence that cooling is significantly more effective when it can be initiated before 3 h. In this review, we propose that given the consistent evidence that milder hypoxic-ischemic

injury is associated with slower evolution of damage, any future clinical trials of delayed treatment starting more than 6 h after an insult should target infants with milder encephalopathy. We then critically examine the evidence that erythropoietin is one of the most promising preclinical candidates either for co-treatment with the

mild therapeutic hypothermia or to support neuroregeneration after the therapeutic window for acute neuroprotection.

Key words : Erythropoietin, hypothermia, neuroprotection, perinatal asphyxia

Introduction : Mild therapeutic hypothermia for full term or near - term infants who develop moderate to severe encephalopathy after perinatal hypoxia - ischemia (HI) is now the standard of care in modern neonatal intensive care units (NICUs). The introduction of therapeutic hypothermia has reduced

adverse outcomes of death or severe disability in this population to approximately 45–50%.^[1] Obviously, a key question is whether there are specific patients or mechanisms that could be targeted to further improve outcomes.

It is highly likely that in part, partial neuroprotection in the clinical practice is related to the formidable clinical difficulties involved in starting hypothermia within the optimal window of opportunity. A recent study found that asphyxiated infants cooled within 3 h of birth had better motor outcomes than when hypothermia was started between 3 and 6 h.^[2] However, in a controlled trial, hypothermia was only started in 12% of infants within 4 h of birth, and the average time of starting was around 5 h of age.^[3] In view of the evidence that earlier cooling is more effective, passive cooling is now often started much sooner after resuscitation, although the effects on outcome are still unclear. Thus, it would be highly desirable to find ways to improve outcome from delayed treatment started either late in the first 6 h, the known window of opportunity for current protocols, or even more than 6 h after an insult.

During the last decade, a range of potential neuroprotective strategies that might be effective when used beyond the first 6 h of life have been tested in animal experiments as well as in small clinical studies. These interventions included inhibition of inflammation, including inhibition of inducible nitric oxide synthase, and stimulation of endogenous repair mechanisms.^[4] In the present review, we will discuss first which patients are most likely to respond to delayed treatment, and then consider the evidence for whether inflammation or erythropoietin (EPO) are potential therapeutic targets.

Milder Injury Evolves More Slowly

Systematic reviews have shown that the beneficial effects of therapeutic hypothermia were greater in infants with moderate than severe encephalopathy;^[5] infants with mild encephalopathy in the first 6 h of life were excluded from most of the randomized controlled trials (RCTs). The definition of moderate encephalopathy is difficult. In many trials, a “modified” Sarnat score was used.

However, this score was developed from a relatively small number of patients at a time when modern neuroimaging was not yet available, and used a mixture of clinical and electroencephalography criteria,^[6] whereas the recent clinical trials only used the clinical criteria. Infants with mild to moderate encephalopathy not surprisingly have less clear-cut symptoms than

those with moderate to severe encephalopathy, and so they may be referred too late to NICUs to qualify for treatment with hypothermia.^[7] In general, hypothermia should start within 6 h after the hypoxic-ischemic insult because of compelling preclinical evidence that it is unlikely to be beneficial after 6 h, and clinical evidence

of improved effect with earlier cooling. Although there is potential for adverse effects when cooling is started late, delayed cooling appears to be safe in an intensive care environment.^[8]

It is important to appreciate that in clinical practice, the severity of neonatal encephalopathy is defined in retrospect, typically after 48 to 72 h. However, for clinical treatment, we need to define severity prospectively. It is less accurate to estimate severity in the first few hours after birth because the clinical severity of encephalopathy evolves over time and the greatest severity attained over time correlates the most with long-term outcomes.^[9] Thus, in some cases, severity

can progress from “mild” to moderate, as described in detail by Sarnat and Sarnat. Given this background, it is not surprising that mild encephalopathy observed in the first few hours after birth can also be associated with adverse long-term effects. However, this relatively neglected

group may actually have the greatest chance of benefit from treatment delayed by more than 6 h after an insult. In neonatal rodents, there is evidence that histological injury evolves more slowly after mild than severe HI. Further, mild hypothermia was protective up to 3 h after

a moderate (90 min) period of HI in P7 rats, whereas hypothermia was not protective after a severe (150 min) insult.^[10] Indeed, 12 h delay after the severe insult before starting hypothermia seemed to increase brain injury.^[10] Other manipulations that increase the severity of the injury, such as pretreatment with lipopolysaccharide, may also be associated with reduced effectiveness of mild hypothermia. Conversely, interventions that delay the evolution of injury, including a brief early interval of hypothermia or some anticonvulsants, such as phenobarbital or topiramate, can expand the window of opportunity for other treatments.^[11] These data suggest the hypothesis that the window of opportunity for delayed treatment would be longer after milder insults.

Blockade Of Inflammation

HI brain injury is consistently associated with a persistent secondary inflammatory reaction. Inflammatory mediators such as tumor necrosis factor- α and interleukin-1 β are induced in rodents within 3 h of HI and peak after approximately 24 h. In the acute phase after neonatal stroke in rats, the inflammatory cells in the brain mainly include resident microglia, rather than invading monocytes.^[12]

The nature of microglial activity evolves dramatically over time after injury.^[13] Thus, whereas activated microglia initially express a so-called M1 phenotype, and can contribute to brain injury through the production of pro-inflammatory mediators, proteases, nitric oxide, and reactive oxygen species, they transit toward a protective or M2 phenotype, with restorative and neuroprotective activity over time.^[14] Consistent with this phenotypic transition, the net effect of depletion of microglia was to worsen injury after HI in neonatal rodents. Nuclear factor - κ B (NF- κ B), an important modulator of inflammation that also controls transcription of genes promoting apoptosis and other cellular injury response genes, is induced maximally in two phases, at 3–6 and 24 h after HI.^[15] Selective NF- κ B inhibition has neuroprotective effects that are predominantly mediated via anti - apoptotic mechanisms, including prevention of accumulation of p53, independent of cytokine production. The timing of NF- κ B inhibition was critical for neuroprotection, which was only observed when early activation was blocked, whereas prevention of both early and late NF- κ B-activity aggravated damage.^[15]

Thus, taken together, these data show that blocking inflammation is extremely unlikely to be an effective strategy for delayed neuroprotection, and indeed may have potential to increase damage by impairing the activity of beneficial microglial phenotypes.

Can We Improve Neuroregeneration? A Possible Role For Erythropoietin: EPO, a hematopoietic cytokine, is an autocrine and paracrine hormone of the nervous system. Neuronal mRNA expression of EPO and the EPO-receptor are markedly increased in response to hypoxia,^[14] likely mediated through the activation of hypoxia - induced - factor -1 α . EPO has both neuroprotective as well as neuroregenerative effects.^[3] Neuroprotection by EPO includes anti - inflammatory, anti - excitotoxic, antioxidant, and anti - apoptotic effects, as well as upregulation of neurogenesis, oligodendrogenesis, and angiogenesis.

Safety Of Erythropoietin In Preterm Infants : EPO is already in wide use to support erythropoiesis in preterm infants, typically in regimes of 400 U/kg 3 times a week subcutaneously, or 200 U/kg daily intravenously.^[3] In controlled trials in preterm infants, three intravenous infusions of up to 2500 U/kg per infusion 24 h apart, or 3000 U/kg at 3 to 6, 12 to 18, and 36 to 42 h after birth,^[15]

respectively, were not associated with any increase in morbidity or mortality.

Clinical Trials Of Erythropoietin In Full Term Infants With Perinatal Asphyxia : In a phase one, dose escalation study in full term infants with perinatal HI, up to 2500 U/kg of EPO per dose starting within 24 h after birth, given up to 6 times, 48 h apart, appeared to be safe.^[16] Two RCTs of EPO for human infants with HI encephalopathy have been published, and one with open label EPO.^[17] Only one study had sufficient power to show beneficial effects of EPO in infants with moderate encephalopathy.^[16] This was a relatively small pilot study that was not powered to assess neurodevelopmental outcomes; 59 infants were studied, of whom 22 children were treated with EPO, 20 controls survived and had neurodevelopmental testing. Further, only two postoperative doses were administered 24 h apart; it is possible that more prolonged treatment is needed for long-term benefit. In a study in term infants who had ischemic infarcts confirmed on magnetic resonance imaging, EPO administration appeared to be safe.^[18] Additional studies of EPO in combination with hypothermia are ongoing, with promising preliminary results.^[16]

This extensive clinical experience with EPO, and evidence that high dose EPO is safe even in sick infants, raises the interesting possibility that an alternative strategy might be to start treatment with EPO in infants with suspected encephalopathy as soon as possible after birth, while they are being evaluated. The decision whether or not to start active hypothermia, and to continue EPO treatment, could then be made once the diagnosis was established, thus minimizing treatment delay. To the best of our knowledge, no preclinical or clinical studies have evaluated the feasibility or net effect on outcomes of such an approach; this would be an important area for future research.

Conclusions : Initiating treatment for neonatal encephalopathy shortly after birth remains a formidable challenge. Although further studies are needed, based on the current evidence, EPO is one of the most promising preclinical candidates either for co-treatment with mild therapeutic hypothermia or to support neuroregeneration after the therapeutic window for acute neuroprotection. We speculate that clinical trials of delayed therapy should consider targeting infants with milder encephalopathy.

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