There has been much progress in understanding the pathogenesis of hypoxic-ischemic brain injury in the near-term and term infant. Although gaps in our knowledge base persist, advances over the past two decades have led to the development of specific brain oriented therapies directed at critical events contributing to tissue damage. The primary goal of these interventions is to prevent or attenuate neurologic and developmental sequelae of brain injury. Examples of current potential treatments include modest reductions in brain temperature, receptor antagonists of excitatory neurotransmitters, reductions in O₂ free radicals, blockade of inflammatory mediators, and inhibition of apoptotic pathways. At present, some of these treatments have sufficient animal data that demonstrate benefit, to justify moving experiments from the laboratory to the clinical arena. Modest hypothermia represents the neuroprotective intervention that has been investigated in the most complete fashion for the newborn, and there are multiple ongoing clinical trials testing its efficacy. This review will address specific challenges that are pertinent to the evaluation of any neuroprotective therapy implemented shortly after birth. Specific issues to be covered include the therapeutic window, establishing a diagnosis of hypoxic-ischemic encephalopathy, patient selection, characteristics of an effective therapy, safety considerations, appropriate outcome variables, and sample size considerations. Since clinical trials of brain hypothermia are in progress, many of these issues will be addressed from the perspective of this specific intervention.

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Neuroprotection for infants at risk for brain injury appears to be one of the most innovative and promising therapies to be introduced in the coming decade. The challenges that are found with this intervention are reviewed in this article.

Therapy as Protection or Resuscitation

One of the major challenges in successfully implementing a neuroprotective therapy is the time of initiation of the intervention. In general, neuroprotective therapies are more effective when used as protection rather than resuscitation. A protective therapy is a treatment that is initiated before a potential adverse event, and thus requires knowledge of the time when a patient would be at high risk for a cerebral insult. For example, infants undergoing cardiopulmonary bypass surgery for correction of complex congenital heart disease represent an ideal population for implementation of treatment as protection. A resuscitative therapy is a treatment that is initiated after a potential adverse event because the possibility for a cerebral insult could not be anticipated. A resuscitative strategy is applicable to the term infant who has incurred a hypoxic-ischemic interval proximate to delivery. Although the incidence of peri-partum hypoxia-ischemia is difficult to estimate, the relative infrequency (approximately 1-2 cases/1,000 live births)¹ ² and unanticipated nature of the responsible events, make detection prior to hypoxia-ischemia usually impossible. Even when there is a high index of suspicion for clinically important hypoxia-ischemia prior to delivery (eg, possible uterine rupture or abruptio placenta), time constraints limit initiation of therapies in a clinical setting. Although less common, unanticipated catastrophic hypoxic-ischemic events may occur following delivery (eg, tension...
pneumothorax, obstructive airway lesions) and would be amenable to a resuscitative strategy.

Given that neuroprotective therapies for the term newborn will need to be initiated as resuscitation, the goals should be to initiate the intervention in as short a period of time following the putative hypoxic-ischemic event. The interval of time after an hypoxic-ischemic event for which a neuroprotective intervention can be implemented with demonstrable efficacy is the therapeutic window. In general, there is decreasing efficacy of neuroprotective treatments the further into the therapeutic window treatment is started. For clinicians caring for critically ill infants following peri-partum hypoxia-ischemia, there are important practical considerations that temper when a brain oriented therapy can be introduced. Relevant to hypoxia-ischemia, initial management needs to prioritize stabilization of the airway if intubated, adjustment of ventilator support, establishment of intravenous and/or arterial vascular access, correction of acid-base disturbances, insurance of adequate perfusion pressure, and maintenance of a normal blood glucose concentration. Once the infant is adequately stabilized, attention needs to be focused on establishing a diagnosis of hypoxic ischemic encephalopathy, and determining if the severity warrants exposure to the risks associated with the potential therapy. Thus, the sequence of stabilization followed by evaluation delays initiation of brain specific treatments, but probably insures a more accurate neurologic assessment since the latter often changes over the first hours after birth.

**Evidence for a Therapeutic Window**

There is sound experimental evidence using animal models that a therapeutic window exists for perinatal animals. At present modest brain cooling is the most effective intervention for hypoxia-ischemia, and has thus led to its use to examine the therapeutic window. Although the number of studies that show the presence of a therapeutic window in the perinatal period is limited relative to adult investigations, the available studies are extremely well done and convincing. The best studies addressing the therapeutic window were performed by Gunn et al and used in-utero sheep undergoing brain ischemia to test the therapeutic efficacy of brain cooling. Cerebral hypothermia was achieved with a cooling cap and was initiated at 1.5, 5.5, or 8.5 hours after ischemia and maintained for 72 hours. The degree of brain cooling is difficult to judge since dura temperature was monitored, but temperature gradients with this mode of brain cooling insure that the outermost portion of the cortex was the coolest. Cooling initiated at 1.5 hours after ischemia reduced brain cytotoxic edema, improved preservation of electroencephalogram (EEG) activity, and decreased the extent of neuronal injury on histologic examination. Benefit was also found when cooling was initiated at 5.5 hours after ischemia but the magnitude of the effect was attenuated.

When brain cooling was initiated at 8.5 hours after ischemia, a time when postischemic seizures commence in this model, neuroprotection could not be demonstrated. Wagner et al, used 7 day rat pups undergoing hypoxia-ischemia to show that a 7 °C reduction in brain temperature initiated 2 hours after hypoxia-ischemia and maintained for 26 hours resulted in brain tissue rescue. The results are important since the presence of a therapeutic window is extended to another species, and the neurologic benefit (demonstrated by both magnetic resonance imaging [MRI] and behavioral assessments) is demonstrable at a time remote from the hypoxia-ischemia (42 days).

Specific characteristics of how cerebral hypothermia is used will also influence the presence and duration of the therapeutic window. For example, the duration of the therapeutic window can be lengthened if the interval of postischemic brain cooling is increased. Consistent with this are the reports of Gunn et al noted above, and the observations from newborn swine that a post-ischemic interval of only 1 hour does not result in neuroprotection even with a short delay before cooling. From the available literature, it appears that a duration of brain cooling between 24 to 72 hours is necessary. Also evident from the available studies is the concept that cerebral hypothermia is more effective the earlier it is started after hypoxia-ischemia. However, a recent study by Taylor et al differs from this concept. Specifically, 14 day rat pups were used to examine 6°C brain temperature reductions when initiated at intervals of 2, 4, or 6 hours after hypoxia-ischemia and maintained for only 6 hours. Surprisingly, cooling initiated...
at 6 hours postinsult led to a better outcome compared to cooling initiated earlier. The authors speculate that there may be a window of time following hypoxia-ischemia when hypothermia affects the most critical pathways, and thus obviates the need for an earlier start and longer interval of cooling. Although provocative, these observations need to be confirmed.

Direct evidence to support the presence of a therapeutic window in human infants at term does not exist. The best available evidence to suggest the presence of a therapeutic window is the observations of Hope et al.10 These investigators examined cerebral energy metabolism using sequential P31 magnetic resonance spectra of brain from 10 infants with birth asphyxia. Six of the 10 infants were studied in the first 24 hours of life and their spectra were similar to normal infants; subsequently, all infants demonstrated changes in high energy phosphorylated metabolites (principally reductions in phosphocreatine and elevations in inorganic phosphorus). The observation that changes in cerebral energy state occur after some interval after birth asphyxia suggests that there is a window of time when neuroprotective therapies could be initiated and be effective.

**Selection of Patients for Neuroprotection**

Translating preclinical studies to the clinical setting is challenging because of the following: 1) Animal models vary in cortical, white matter and deep gray matter structural development when compared with the human neonate; 2) Postinjury rescue treatment seems to be the only feasible approach with acute perinatal asphyxia; 3) The potential window of rescue neuroprotection in perinatal animal models seems to be 6 hours after a timed injury; 4) In the human neonate, neither the timing nor the severity of the hypoxic-ischemic injury is known; and lastly a single therapeutic agent or approach may impact only 1 step or steps in the cascade of events after a hypoxic-ischemic event; therefore a multitreatment approach may be needed.

Early identification of acute encephalopathy is crucial in view of the therapeutic window and identification of infants at high risk for neurological morbidity needs to be accurate to minimize the risk of potential therapies. Encephalopathy, which generally evolves over 2 to 3 days, is the best predictor of long-term outcome. A combination of abnormal physiologic and biochemical markers have been used with or without encephalopathy in the immediate newborn period to identify infants at risk for brain injury. The most common parameters used in newborn assessments are as follows:

**Apgar score.** Very low and persistently low Apgar scores are associated with a high mortality rate and cerebral palsy in survivors. Moderately low scores and scores that improve between 1 and 5 minutes do not correlate with later neurological outcome.11 Apgar scores are lower in neonates with seizures, compared to controls.12

In a study evaluating the relationship of Apgar score to encephalopathy, a score of 0-3 at minute 5 was significantly related to encephalopathy.13 However, a low Apgar score may reflect not only hypoxia-ischemia, but also lack of tolerance to labor by a fetus compromised by prenatal disease, medications received by the mother, fetal malformations, and neuromuscular and cardiopulmonary disease in the neonate.14

**Fetal heart rate patterns.** No specific pattern of fetal heart rate abnormalities has been found that is predictive of neurological abnormalities.15,16 Multiple late decelerations and lack of beat-to-beat variability of the fetal heart rate pattern are associated with an increased risk for cerebral palsy.17 Term infants with encephalopathy have been shown to have absence of fetal heart rate accelerations and low variability compared to control infants with no encephalopathy.18 However, these findings have low sensitivity and specificity.

**Acid base status.** An association between umbilical artery pH <7.0 and low 1 and 5 minute Apgar scores, neonatal seizures and death has been noted in a large series of consecutively studied term infants.19 Mixed or metabolic acidosis, rather than respiratory acidosis, appears to correlate with complications in the neonate.20 Neonatal morbidity has been shown to increase with every 0.10 decrement in pH.21 A pH of 7.0 appears to reflect the degree of acidosis below which end organ dysfunction is detected among term infants with fetal distress and newborn depression.22 Umbilical arteriovenous pH, pCO₂ and pO₂ differences have been correlated with neonatal morbidity and neurologic injury in term infants with perinatal asphyxia.23 On the other hand, term and near term infants born
with an umbilical arterial pH ≤7.0 who are clinically stable at birth and are admitted to a non-intensive care setting do not have manifestations of hypoxia-ischemia in the newborn period. Unanticipated acidemia in stable neonates may be associated with induction of labor and epidural and spinal anesthesia. Furthermore, acidemia at birth may reflect underlying disorders, including sepsis. Thus, pathologic fetal acidemia alone does not accurately identify newborns with hypoxic-ischemic injury.

**Neurological signs.** In term infants, neurological signs following acute asphyxia have been characterized by Sarnat and Sarnat as hypoxic-ischemic encephalopathy. Signs of central nervous system injury evolve over 72 hours following the insult with seizures occurring usually within 12 hours or hypotonia in the immediate neonatal period. Neurodevelopmental outcome has been correlated with the stage of encephalopathy. The risk of death is 5% after moderate encephalopathy and 25%-60% after severe encephalopathy. The risk of severe handicap in survivors is 20% after moderate, and 50%-100% after severe encephalopathy. The risk of cerebral palsy after obstetrical complications is increased in premature infants with data available within 4 hours of birth with a sensitivity of 1.0, specificity of 0.82, a positive predictive value of 0.85 and a negative predictive value of 1.0.

**Neurophysiologic markers of brain injury.** Electrical background activity is more predictive of outcome in infants than the presence or absence of seizure activity. However, conventional multichannel recordings are too cumbersome and are not readily available in the clinical setting. The amplitude integrated EEG (aEEG) is a simplified form of continuous EEG monitoring that gives an overall impression of cerebral cortical activity. The aEEG records a single channel from biparietal electrodes and is easy to apply at the bedside. There is good correlation between the aEEG and conventional EEG. In studies evaluating aEEG and outcome, descriptions of aEEG have included both minimum and maximum voltage and the pattern of the aEEG. The aEEG patterns have been grouped into 5 categories as follows: 1) Continuous normal voltage pattern (CNV) with maximum voltage 10-50 μV with periods of increased variability due to sleep; 2) Discontinuous normal voltage (DNV) with more continuous normal voltage with periods of intermittent low voltage; 3) Discontinuous background pattern of burst suppression (BS) with periods of very low voltage intermixed with bursts of higher amplitude; 4) Continuous low voltage (CLV) around or below 5μV; and E) Very low voltage with activity below 5μV, known as flat tracing (FT). A CNV aEEG pattern is predictive of good outcome, and almost all asphyxiated full term infants with FT patterns either die or develop sequelae. Al Naqeeb et al classified the aEEG background in neonates with encephalopathy in a more simplified manner that included normal amplitude (upper margin of band of aEEG activity >10μV and lower margin >5μV), moderately abnormal amplitude (upper margin >10 μV and lower ≤5 μV), and suppressed amplitude (upper margin <10μV and lower margin <5 μV). The aEEG appears to be a good predictor of neurodevelopmental outcome for the infants studied within 12 hours of birth with a sensitivity of 1.0, specificity of 0.82, a positive predictive value of 0.85 and a negative predictive value of 1.0.

The efficacy of measuring cerebral function within the first 6 hours of life by aEEG was...
demonstrated in a study of 47 infants by Hellström-Westas.\textsuperscript{45} The ability to predict a normal outcome after a normal tracing or a poor outcome with an extremely low voltage reading were 91%, although the burst suppression pattern was less predictive. The predictive value of the aEEG is better at 6 hours of age than at 3 hours in studies evaluating outcome from 1-6 years.\textsuperscript{43} At 3 hours, the sensitivity and specificity of the BS pattern together with FT and CLV was 85% and 77%, respectively, while at 6 hours it was 91% and 86% respectively. Using the criteria at 3 hours for a neuroprotective intervention would result in unnecessary treatment in a small percentage of cases; on the other hand, some infants would not be offered treatment.\textsuperscript{46}

The pitfalls and caveats encountered with aEEG monitoring have been reviewed by Hellström-Westas.\textsuperscript{47} Artifacts can occur during the study readings with a loose electrode, gasping respirations, high-frequency ventilation, or non-optimum head position. Readings may be dampened because of a subgaleal hemorrhage or may be asymmetric due to intraparenchymal infarcts. Groenendaal and de Vries\textsuperscript{46} suggest pattern recognition requires experience and they do not advocate the use of absolute values alone.\textsuperscript{44}

Recently, Shalak et al\textsuperscript{48} showed that aEEG coupled with an early neurological examination enhances prediction of term infants at risk for persistent encephalopathy. Among 50 term infants with clinical and biochemical criteria of acute intrapartum distress, an abnormal aEEG using criteria of al Naqueeb\textsuperscript{44} had 89% specificity, 79% sensitivity and 73% positive predictive value. An abnormal neurological examination had 78% specificity, 78% sensitivity and 58% positive predictive value; the combination of neurological examination and aEEG had specificity of 94% and positive predictive value of 85%.

Other early markers of acute intrapartum asphyxia. Cerebrospinal fluid (CSF) levels of interleukin 6 (IL-6) obtained within 8 to 90 hours of life have been shown to be elevated in neonates with brain damage (imaging or pathological evidence) after perinatal asphyxia or encephalopathy;\textsuperscript{49,50} In addition, IL-8 was also related to the degree of hypoxic-ischemic encephalopathy (HIE).\textsuperscript{49} CSF levels obtained within 6 hours of birth (potential window of therapy) have shown elevated interleukin 1β and tumor necrosis factor α levels that correlated with outcome.\textsuperscript{51} Another early marker that has correlated with hypoxic-ischemic encephalopathy is an elevated urinary lactate to creatinine ratio evaluated within 6 hours of age by proton nuclear magnetic resonance spectroscopy. The ratio of urinary lactate to creatinine ratio was also significantly higher in infants who had adverse outcomes at one year.\textsuperscript{52} These markers may not be available in a clinical setting.

The selection of neonates for neuroprotective therapies is very challenging; using one set of criteria (5 min Apgar <6, blood pH of 7.00 or less, and need for intubation and ventilation in the delivery room)\textsuperscript{36} applied in a retrospective manner to a population, Nelson and Grether\textsuperscript{53} showed that most infants eventually diagnosed with CP had nonasphyxial conditions that may have contributed to adverse neurological outcome. These conditions include maternal or neonatal infection,\textsuperscript{33,54} abnormal coagulopathy secondary to factor V Leiden mutation\textsuperscript{55,56} or anticardiolipin antibodies in blood of mothers.\textsuperscript{57} However, it remains unknown whether the same criteria applied prospectively in the neonatal period would identify a high percentage of all infants in a population with hypoxic-ischemic injury.

Although there are many studies which have examined the relationship between prenatal and perinatal events and neurodevelopmental outcome, the largest database on this topic is derived from the National Collaborative Perinatal Project (NCPP) with enrollment of more than 50,000 mother-infant dyads between 1959 and 1966. A high mortality rate occurred among term infants with severe asphyxia, so relatively small numbers of children with low Apgar scores were evaluated at 7 years.\textsuperscript{11} If these children had survived, a stronger relationship between perinatal asphyxia and poor outcome may have been found.\textsuperscript{58} Given the advances in Obstetric and Neonatal care, the cluster of perinatal events associated with neonatal neurological dysfunction noted in NCPP\textsuperscript{52} need to be re-examined in a current data set. In view of these constraints, the ongoing trials of modest hypothermia are using a combination of criteria to establish a diagnosis and identify study candidates. These criteria include indicators of an acute event (fetal acidemia or resuscitation after a sentinel event around the time of delivery), and evidence
of an acute encephalopathy. Some trials are also employing the aEEG to confirm the clinical impression of encephalopathy.

**Characteristics of an Effective Therapy for Neuroprotection**

There are a number of important characteristics for an intervention to be successful as a possible brain oriented therapy. This includes targeting specific mechanisms mediating the pathogenesis of tissue injury, ease of initiation within a limited interval of time, maintenance of the therapy for the necessary interval of time to optimize the neuroprotective effect, and absence of frequent and/or serious adverse events associated with the therapy. Each of these points will be briefly addressed.

**Targeting specific mechanisms.** Although the pathogenesis of peri-partum brain injury is extremely complex and not completely understood, the initiating event often involves hypoxia-ischemia. Hypoxemia alone probably has relatively little adverse effects on the brain provided that perfusion pressure is maintained. This reflects the typical homeostatic adjustments in cerebral blood flow characteristic of mammalian species. The latter maintains bulk O\textsubscript{2} delivery to the brain, and consequently brain O\textsubscript{2} consumption. Similarly, ischemia without alteration of respiratory gases (ie, alterations in perfusion pressure and blood flow only) is a relatively uncommon event in the term infant and most often occurs in the absence of an identifiable precipitating event or condition. Rather it is the combination of hypoxia and ischemia that initiates a complex cascade of events contributing to brain injury. Although the proximate cause for hypoxia-ischemia is evident for some infants (eg, impaired placental gas transport due to an abruption, cord accident), the association of brain injury with inflammatory conditions suggests that triggering pathways to injury may be more complicated than previously thought.

Given these caveats, brain energy failure after hypoxia-ischemia is associated with 2 phases of pathologic events that culminate in brain injury. These phases have been well described in newborn animals and are termed primary and secondary energy failure based on characteristics of the cerebral energy state used to describe the temporal sequence. The tie between hypoxia-ischemia and brain injury is the initiation of multiple mechanisms contributing to injury, with varying links between mechanisms. Important pathways contributing to perinatal brain injury include excessive exposure to excitotoxic neurotransmitters, free radical damage, inflammation, and excessive apoptosis. Other mechanisms such as defective osmoregulation and inhibition of protein synthesis have been addressed in adult but not perinatal animals.

Ideally prevention of hypoxia-ischemia is the most effective strategy for this form of brain injury, but is usually not feasible under real world conditions. Neuroprotective strategies thus need to focus on downstream events from the putative hypoxia-ischemia. Neuroprotective therapies can either be specific or nonspecific with respect to a mechanism that prevents or attenuates the extent of brain injury. An example of a specific neuroprotective therapy is MK-801. This drug noncompetitively blocks the N-methyl-D-aspartate receptors and effectively inhibits the neurotoxicity associated with excessive release of excitatory neurotransmitters during hypoxia-ischemia. Another example is the use of rhIL-1ra to protect against cerebral infarction after hypoxia-ischemia. The latter is an interleukin-1 receptor antagonist and supports the concept that inflammatory mediators play an important role in the pathogenesis of hypoxic-ischemic brain injury. Although specific neuroprotective treatments are efficacious in the experimental setting of hypoxia-ischemia, it remains unknown if one pathway is more predominant than others after hypoxia-ischemia in human infants. Furthermore, characteristics of the infant, such as genetic predisposition, growth parameters, or preconditioning, are likely to influence which mechanistic pathway(s) has the greatest influence on outcome.

Until there is a better understanding of the interplay among the multiple mechanisms of injury for specific clinical conditions, a good rationale could be made for the use of a non-specific neuroprotective therapy. The latter strategy would involve treatments that affect multiple mechanistic pathways contributing to brain injury. Modest brain hypothermia is the prototypical example of a non-specific neuroprotective therapy. A relatively small reduction in brain temperature (1-6°C) of neonatal animals is associated with better maintenance of the
cerebral energy state during and immediately after ischemia, attenuation of the release of excitatory neurotransmitters and decreased caspase-3 activation and morphologic evidence of apoptosis. Other neuroprotective effects of cerebral hypothermia have been shown in adult animals and include normalization of the decrease in protein synthesis, reduction of free radicals and modulation of microglial activation and cytokine production. The net effect of modest hypothermia on multiple pathways is an attenuation of secondary energy failure and the neuroprotective benefits previously cited. These considerations also raise the possibility that combinations of therapies (non-specific and/or specific treatments) may be a reasonable approach for future clinical trials.

Ease/time of treatment initiation. An important aspect of neuroprotective therapies is the time window available to initiate treatment. Although the duration of the therapeutic window is not precisely known for human infants, animal data summarized above suggests that it is in the order of hours. Thus, treatment needs to be initiated in a short interval of time. Intravenous pharmacological therapy is attractive because of the rapidity and ease of medication administration. A potential limitation to pharmacological therapy is that therapeutic concentrations must be rapidly achieved throughout the brain tissue. The blood-brain-barrier has always posed a potential limitation to the effectiveness of drug therapy for the central nervous system. Traditionally, development of new drug therapies has focused on the drug-activity relationship, ie, the drug-receptor interaction, and there has been markedly less attention to the structure-transport relationship, ie, membrane permeation. Carriers will be needed to insure transport across the blood brain barrier unless compounds are lipid soluble and of low molecular weight. An additional concern for the newborn is that maturational differences will prohibit ready extrapolation of experiences from adults to neonates.

In contrast, modest hypothermia represents a therapy that can be implemented easily, rapidly, and the extent of brain temperature reduction can be assessed. In pilot animal studies performed for the National Institute of Child and Human Development (NICHD) clinical trial of whole body hypothermia for perinatal asphyxia, the implementation of a cooling regimen and relationship between systemic and brain temperature was evaluated. Three miniature swine were instrumented and ventilated, and temperature probes were placed in the esophagus and the brain (1 cm and 2 cm beneath the parietal cortical surface and on the dura). Body cooling was achieved by having the animals lay on a cooling blanket interfaced with a commercially available cooling system (Blanketrol II Hyper-Hypothermia system, Cincinnati Sub-Zero Products, Cincinnati, OH). This system allows for temperature control using an automatic control mode (servo), and the esophageal temperature was servo regulated between 38-39°C during baseline conditions. During whole body cooling esophageal temperature was servo controlled to 33.5°C, and the target temperature was reached within 90 min of initiation of whole body cooling, and maintained for up to 6 hours. The table shows the difference in temperature between the esophagus and each brain region assessed. The small difference in temperature between the esophagus and the brain at a 2 cm depth indicates that the esophageal temperature is an excellent index of core brain temperature during normothermic and hypothermic conditions, and that whole body hypothermia cools the entire brain. Furthermore, the servo control mechanism provided automatic adjustments in temperature of the cooling blanket to maintain esophageal and thus brain temperature at the set point.

The animal observations were used as the methodological basis for a pilot study of either normothermia or modest hypothermia in term infants with encephalopathy at birth. The target esophageal temperature was 34.5°C and was achieved within 30 minutes, followed by an overshoot of 1-1.5°C, with stabilization of the esophageal temperature at the set point by 90 min of cooling (see Fig 1). Preliminary analysis of the main trial (esophageal temperature set point of 33.5°C) indicates that achievement of the target esophageal temperature also occurs within 90 min. The Blanketrol Hyper-Hypothermia (Cincinnati Sub-Zero Products) system is simple to use, effectively lowers body and brain temperature, and requires no adjustments from the bedside provider.

Maintaining the neuroprotective intervention. The duration that a neuroprotective intervention needs to be continued for optimal efficacy re-
mains uncertain. What appears to be certain is that progression to secondary energy failure signifies irreversible development of brain injury. In neonatal animals secondary energy failure occurs between 24 to 48 hours after hypoxia-ischemia. Furthermore, study of brain ischemia in fetal sheep has delineated the temporal sequence of the accumulation of neurotransmitters and nitric oxide in the extracellular space, EEG activity, and cortical impedance. After the acute changes of ischemia have subsided, there are prolonged elevations in excitatory neurotransmitters, indices of nitric oxide synthesis, and decreases in cerebral impedance, suggesting that treatment may need to be continued for up to 72 hours.

If pharmacological interventions are used to achieve neuroprotection, the pharmacokinetics to maintain therapeutic concentrations in the brain tissue for some interval of time will need to be understood, in addition to transport across the blood-brain-barrier. Hypoxic-ischemic involvement of organs such as the kidney and liver may affect the pharmacokinetics. Cerebral hypothermia is an appealing therapy that obviates many of these issues. The use of esophageal temperature to guide brain cooling provides a convenient, minimally invasive, and reasonably accurate index of core brain temperature (Table 1). Results from the NICHD Neonatal Network pilot study of whole body cooling (Fig 1) showed that esophageal temperature could be controlled to a desired target temperature (34.5°C for the pilot) and maintained constant over 72 hours using the servo control mode of the Blanketrol Hyper-Hypothermia system (Cincinnati Sub-Zero Products). There are some important caveats to these observations when monitoring cerebral hypothermia that are dependent upon the mode of brain cooling. If brain hypothermia is achieved by cooling the body, the brain is uniformly cooled with minimal temperature gradients from the core to the periphery, episodes of hypoxia do not alter brain temperature, and systemic temperature is a good index of brain temperature. In contrast, if brain hypothermia is achieved by selectively cooling the head, there are prominent temperature gradients from the warmer core to the cooler periphery, hypoxia does alter brain temperature, and a single systemic temperature site cannot provide a valid index of brain temperature. Finally, if the clinical course is complicated by brain ischemia, systemic temperature will not be an accurate index of brain temperature irrespective of the mode of brain cooling.

Safety of the neuroprotective intervention. The issue of safety is pertinent to any neuroprotective intervention irrespective of the mechanism of action. The ideal treatment would be efficacious with minimal adverse effects. Efficacy and safety are usually not completely appreciated for new treatments undergoing clinical trials, because clinical experiences that form the basis for a definitive clinical trial are often cohort studies or small trials without the power to adequately assess outcome. Thus, entry criteria for the evaluation of any new therapy in sick infants needs to identify the group at highest risk for brain

![Figure 1](image-url)  
(A) Temperatures of the esophagus and (B) abdominal skin are plotted for the normothermic group (open triangles) and the hypothermic group (closed circles). Values are mean ± SD. Normothermic infants were servo controlled at 36.5°C using the abdominal skin as the control site, and the hypothermic infants were servo controlled at 34.5°C using the esophageal temperature as the control site. The hypothermic group was rewarmed after 72 hours of cooling.

| Table 1. Temperature Difference Between the Esophagus and Specific Brain Sites |
|----------------------------------------|-----------------|
| **Temperature Difference (°C)**       |                 |
| Esophageal - Brain 2 cm               | 0.1 ± 0.3*      |
| Esophageal - Brain 1 cm               | 0.4 ± 0.5       |
| Esophageal - Dura                     | 1.1 ± 0.9       |

*P < .001 for esophageal-Brain 2 cm vs esophageal - Brain 1 cm and esophageal - Dura.
injury to justify potential therapy associated risks. Therapies such as modest hypothermia and high dose barbiturates are attractive from this perspective since there is an existent knowledge base regarding potential adverse effects. Unless a therapy is recognized to have great hazards from initial experiences, a randomized clinical trial represents the optimal way to evaluate efficacy relative to complications. In this regard, randomized multicenter trials should be monitored by a Data Safety Monitoring Committee with safety and outcome data evaluated at multiple intervals. Methods such as that of O’Brien and Fleming permit multiple looks at a fixed sample size while maintaining overall type 1 error rate, and sacrificing little in terms of power.

**Role of neuroimaging in trials of neuroprotection.** The value of CT scanning as a good prognostic tool to evaluate subsequent neurological sequelae is limited. Sensitivity and interobserver agreement are better with MRI and computer tomography rather than with sonography for detection of ischemia or infarction. MRI is now emerging as a more sensitive and early indicator of brain injury in neonates than computer tomography, especially when combined with EEG. Abnormal signal density seen in the posterior limb of the internal capsule had a 100% positive predictive value for abnormal outcome at 1 year of age.

Diffusion weighted MRI shows areas of injury in the basal ganglia and thalami within 6 hours of injury. MRI imaging in the neonatal period can now predict type of CP seen at follow up: children with postero-lateral lentiform nucleus and ventro-lateral thalamic injury developed dyskinetic CP; those with extensive thalamic injury, central region and hippocampal lesions developed spastic CP, while deep grey nuclei and cortico-subcortical lesions in the central region were associated with mixed CP. Proton magnetic resonance spectroscopy (MRS) at 6 days of age in acutely asphyxiated infants correlated well with 30 month outcome. The basal nuclei and intervascular boundary zone NAA/choline ratio were highest in the children with normal outcome and lowest with those with an abnormal outcome; while the lactate to choline ratio was lowest in the normal children and highest in abnormal children. Positron emission testing scanning at 4-28 days of age has now been shown to correlate with neurological outcome; the lowest glucose metabolic rate was seen in the CP group. Thus, imaging of the brain during and/or after neuroprotective interventions will be an important component in the clinical assessment of brain injury. However, there are practical concerns with present technology (logistics of off-site imaging facilities, moving critically ill neonates) that may limit the role of neuroimaging in any trial of brain oriented therapies.

**Primary outcome and sample size considerations.** The primary outcome of a neuroprotective trial should be the combined endpoint of death or moderate or severe disability at an early age: 18 to 22 months is the earliest age at which major disability can be detected. All evaluations should be performed by certified and trained individuals masked to intervention status. Neuromotor disability may be based on the neurologic examination and functional disability by the Gross Motor Function (GMF) classification. Psychometric testing can be based on the Bayley Scales of Infant Development (BSID II; MDI and PDI normal values 100 ± 15, mean ± SD). Auditory and visual outcome should be assessed by specialists masked to intervention status. All outcome variables should be defined a priori.

**Moderate disability** can be defined as Bayley MDI between 1-2SD below mean and one or more of the following: Level 2 on GMF, deafness with no amplification or seizure disorder.

**Severe disability** can be defined as any of the following: Bayley MDI >2SD below mean (<70), GMF level 3-5, deafness requiring hearing aids (>60 dB) or bilateral blindness (<20/200 acuity).

Sample size estimates should be powered to include a 10% rate of loss to follow-up. A reasonable effect size for a favorable neuroprotective effect would be the ability to detect a reduction to 30% from a 50% event rate of death or disability. With a two-tailed type 1 error of 0.05, power of 80%, the sample size is 100 infants per group or 200 total. Multicenter studies are needed because the number of term infants at risk for neurologic dysfunction will be too few in any single institution. The number of infants who suffer irreversible neurologic damage is a subset of infants who are subject to asphyxia at birth; hence large numbers of infants need to be screened.
Duration of follow-up. A critical issue in evaluating efficacy of neuroprotective therapies is the age of follow-up for the outcome of interest. All the current trials evaluating hypothermia as a neuroprotective strategy have the primary outcome as death or disability at 18 months of age. This age is the earliest age at which major disability can be detected. To understand whether hypothermia or any other neuroprotective treatment is beneficial, it is necessary to evaluate relationship of intervention to early childhood outcome because interventions may influence not only major neurological sequelae detected at 18 months, but also potential sequelae of brain injury in childhood-behavior, learning, fine motor development and psychosocial and psychiatric outcomes. For example, the relationship between the degree of cooling in neuroprotective trials and outcome may be discernible only on subtle outcome evaluations beyond 18 months. The expense of tracking infants over long time intervals and the attrition in follow-up are critical issues in determining the duration of follow-up for the primary outcome variable.

There is some experience with long-term outcome after hypoxic-ischemic encephalopathy during early childhood. At 5 years of age, surviving infants with moderate or severe encephalopathy tend to have either a normal gross motor exam or severe disability. In this study, fifty percent of survivors were multiply handicapped with spastic quadriplegia, persistent seizure disorder, microcephaly and language delays. Only 9 of 24 survivors had a McCarthy General Cognitive Index (GCI) that was normal. Lack of head growth at 3 months and persistent seizure disorder correlated with GCI and verbal, perceptual performance, quantitative, memory and motor subscores <2SD below the mean. Cordes also has showed that serial head measurements during the first few months in term infants with HIE predicts later microcephaly.

There are limited data on school age outcomes of nondisabled survivors. Robertson et al focused on findings among children who survived moderate neonatal encephalopathy with gross-motor ability intact. Children from 2 birth cohorts, 1 born 1974-1979 and the other born 1982-1986 were followed to 8-10 years and compared to matched control groups. The frequency of disabilities (8% following moderate HIE and 100% following severe HIE) was unchanged between the 2 time periods. There were no survivors without a disability following severe neonatal encephalopathy. The nondisabled survivors of moderate neonatal encephalopathy had a significantly lower percentage achievement to age for motor tasks including everyday motor (walking, running), complex motor (hopping, balance) and fine motor tasks. Academic achievement (reading, arithmetic) greater than one level below expected grade level of comparison children was seen to be significantly greater among nondisabled survivors of moderate neonatal encephalopathy and full scale, verbal and performance IQ was also significantly lower in the nondisabled survivors of moderate neonatal encephalopathy in both birth cohorts.

Undertaking long-term outcome such as school age follow-up is ideal in evaluating brain oriented therapies. If loss to follow-up can be minimized, important issues to be considered are the use of evaluation tools that allow comparison of outcomes across studies. This will include assessments of neurologic status and classification of impairments. Imaging studies should be obtained at the early childhood examination. Domains to be tested in the children should include verbal and performance IQ, attention, executive function, and visual and perceptual motor skills. In addition, parental IQ and family support should be assessed. Last, the cost-effectiveness of the intervention should be examined.

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