Neonatal Hypoxic-Ischemic Brain Injury: Mechanisms and Strategies for prevention and Treatment

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Most infants affected by hypoxic-ischemic (H-I) brain injury do not require surgical intervention. However, some of these infants as well as infants with certain congenital anomalies that place them at risk for H-I brain injury will present for surgical intervention. The incidence of H-I injury is estimated to be between 1 and 6 per 1000 live term newborns (Ferriero, 2004). Neonatal hypoxic-ischemic brain injury remains the major cause of long-term morbidity, in the form of motor and cognitive delays. The nature of the deficits is dependent on the gestational age and severity of the insult.

Prospective and retrospective studies have attempted to identify infants at risk for hypoxic-ischemic encephalopathy. At risk infants include premature and term infants with low 5 min Apgar scores (<5), low cord blood pH (<6.90), need for intubation and/or CPR and a blood glucose <40 mg/dl (Shalak and Perlman, 2004). The incidence of overt neonatal encephalopathy is estimated to be from 1-6 per 1000 term births. Infants can also be placed at risk by the presence of congenital anomalies that disturb cerebral blood flow and oxygenation. A recent study of infants with congenital cardiac defects found that a subset of infants had reduced cerebral blood flow that was not responsive to increasing pCO₂. These infants are at risk for H-I injury.

Pre-term infants suspected of having sustained a H-I insult can be graded both clinically and through neuro-imaging. Several clinical grading systems exist that allow infants to be classified as having mild, moderate or severe injury (see Ferriero, 2004 for example). Scoring correlates with early mortality and neuro-developmental outcome at 30 months. Among all infants with H-I injury there is a 15–20% mortality in neonatal period and 25% have permanent neurological deficits. Mortality among the severely affected can exceed 50%; most survivors will have major neurologic deficits.

Neuro-imaging studies have attempted to correlate findings with both early mortality and later neuro-developmental outcomes. Measurement of apparent diffusion coefficients and regional spectroscopy can identify infants at high risk of bad outcomes. In term infants reduced apparent diffusion coefficients in the posterior limb of the internal capsule are prognostic for poor outcomes following perinatal hypoxia-ischemia (Hunt et al., 2004). Lactate accumulation in basal ganglia can presage later structural damage in the same regions.

Mechanisms of Injury:

Hypoxic-ischemic brain injury is not a static process that is complete at the time of insult. The injury evolves over days to weeks and is different from adult ischemic injury. Several types of processes are involved at different stages of the injury; these processes include inflammation, oxidative stress (reactive oxygen species), neuronal production of nitric oxide and excitotoxicity. The picture is complicated by changes in the vulnerability of brain regions and cell types with gestational age. For example, the parietal periventricular area is highly susceptible to periventricular leukomalacia (PVL) between 24 and 32 weeks gestation. Pathology studies in humans have found that pre-oligodendrocytes, rather than mature oligodendrocytes, populate this region in the same time frame (Back et al., 2001). These pre-oligo’s are especially sensitive to oxidative stress and excitotoxicity due to differences in the composition of AMPA type glutamate receptors (Deng et al., 2004) and the level of endogenous anti-oxidants between mature oligo’s and pre-oligo’s.

While PVL is a common lesion following H-I in pre-term infants less than 32 weeks gestation, deep nuclear (basal ganglia and thalamus) and neocortical injury are the hallmarks of H-I in near-term and term infants. Animal studies suggest that brain regions that express neuronal nitric oxide synthase (nNOS) are the same regions that are vulnerable to H-I injury (Ferriero et al., 1996). In the immature brain the nNOS expressing cells are resistant to H-I but their neighbors are killed. As these nNOS containing cells mature the resistance to H-I injury is lost. In mice lacking nNOS cell death and brain injury is reduced. However, if an inhibitor of NOS such as L-nitroarginine is administered to the nNOS deficient mice, the injury is as severe as in wild-type mice. This suggests that endothelial NOS (eNOS) plays a protective role in limiting H-I injury.
The inflammatory pathway has also been found to contribute to H-I injury. The major mediators appear to be the interleukin-1 receptor (IL-1R) (Hu et al., 2005) and the Fas death receptor (Graham et al., 2004). The downstream signaling pathways lead to activation of nuclear-factor kappaB and increased transcription of COX2 and iNOS. IL-6, tumor necrosis factor-alpha (TNFa) and expression are also enhanced. Blockade of the IL-1R within 2 hours of a H-I insult dramatically reduces injury as measured by infact size 7 days after insult. Knockout of functional Fas death receptors leads to reduced injury in cortex but does not protect the hippocampus (Graham et al., 2004). COX2 and iNOS appear to be the agents of tissue injury as TNF prevents or mitigates neuro-degeneration and IL-6 has an independent neuro-protective effect (see Shalak and Perlman, 2004).

In addition to activation of the IL-1R by H-I, the activation of glutamate receptors is a key component of initial and delayed H-I injury. Glutamate receptors fall into two broad groups; ionotropic (iGluR), which are ligand-gated ion channels and metabotropic (mGluR), which are G-protein coupled receptors that modulate K+ channels, GABA receptors and iGluRs. The iGluRs are divided by their affinity for NMDA, AMPA and kainite. Calcium entry through specific pathways rather than total Ca\(^{2+}\) accumulation is the determinant of whether a neuron is injured. Ca\(^{2+}\) can enter through both NMDA and AMPA receptors in neonatal rodents and premature infants due to lack of a specific subunit (GluR2) in the AMPA receptor. This same GluR2-lacking AMPA receptor is also highly permeable to Zn\(^{2+}\), which is toxic to mitochondria and can activate activation of cell death pathways. H-I can modify the adult AMPA receptor in selected brain regions so that it the population of AMPARs includes the GluR2-lacking form.

The main pathway for Ca\(^{2+}\) entry is the NMDA receptor (NMDAR). These receptors are couple to a large complex of scaffolding and cytoskeletal proteins as well as mediators of down-stream signaling pathways. Some pathways are cell survival pathways while others can mediate cell death. Activation of nNOS by Ca\(^{2+}\) entering via NMDARs is an example of a cell death pathway. Blockade of NMDARs as a treatment for H-I has been uniformly unsuccessful due to undesirable side effects.

It is also clear that activation of the inflammatory pathway prior to a H-I insult results in more neuronal injury than either alone. In addition white matter injury may be more expensive when the inflammatory pathway has been activated prior to H-I (Hagberg et al., 2002).

Evolution of the Injury
The initial H-I insult usually results in a necrotic cell death due to energy failure, Na and water accumulation and cell rupture. This then leads to activation of inflammatory cells and release of IL-1 and other cytokines and chemokines. In addition to the necrotic core there is an entire region of neurons that may survive or die depending on balance between pro- and anti-apoptotic factors. This balance is decided during the reperfusion phase and the processes involved last days to weeks. Progenitor cells destined to become new neurons, astrocytes or oligos enter cell cycle in larger numbers than normal following H-I. This happens at the same time as delayed cell death due to pathways activated by the H-I insult. Many of these progenitors will die during the process of differentiation toward final cell fate. The cell fate appears to be regionally specified within the hippocampus. Cells labeled during cell-cycle entry within 6 days of H-I differentiate primarily to astrocytes in the CA1 and to mature neurons in the dentate gyrus. This is summarized in the diagram below:

Prevention and Treatment
The pediatric anesthesiologist may be called on to care for an infant that has experienced or is at high risk for a H-I insult. This group of patients includes newborns with major congenital heart defects. In some cases the infant has already experienced a H-I insult; the objective is then to avoid further injury. Laboratory studies in animals and human trials have shown that hyperoxia and hypocarbia following H-I result in a worse outcome than normoxia and normocapnia. Hyperthermia will also result in a worse outcome. Maintenance of perfusion pressure and avoidance of hypoglycemia, hypocarbia and hyperoxia are important.
The anesthesiologist can be challenged by the conflicting needs for surgical analgesia and hemodynamic stability in the face of a procedure that can result in profound physiologic changes. The choice of anesthetic agent has been complicated by published studies showing neurological injury following exposure to certain anesthetic agents in seven-day-old rats. Anesthetic agents implicated include ketamine, midazolam, nitrous oxide and isoflurane (six hour exposure) (Jevtovic-Todorovic et al., 2003). The injury is attributed to prolonged blockade of NMDA receptors or activation of GABA type A receptors. The 7 day-old rat brain is developmentally equivalent to a 28-32 week pre-term infant (Dobbing and Sands, 1979; Romijn et al., 1991) and the findings may have limited applicability to term infants. There are no data indicating that narcotics such as fentanyl are deleterious. Further, there are data from different animal models indicating that volatile agents may protect the brain of the <insert term> infant from H-I injury (Loepke et al., 2002, McAuliffe et al., 2005).

It is important to remember that maintenance of hemodynamic stability and cerebral perfusion is all-important. The anesthetic technique should be selected with this goal in mind. A narcotic based techniques supplemented with volatile agents may be the good choice in many circumstances. The choice of volatile agent should be dictated by hemodynamic considerations as there are no data comparing one volatile agent to another in terms of neuroprotection in a term newborn model.

Once the diagnosis of hypoxic-ischemic encephalopathy is made clinically usable treatment options are currently limited. Many therapies that appeared promising in animal studies have failed to advance to human trials or have failed in human trials. There are no drugs with an FDA approved indication for treatment of hypoxic-ischemic injury in the newborn period. At present the only modality that has been used in multiple trials is hypothermia. Hypothermia can be used in two ways, either whole body (33.5°C for 72 hours) (Shankaran et al., 2005) or selective cerebral hypothermia with moderate systemic hypothermia (34-35°C for 72 hours) (Gluckman et al., 2005). Sinus bradycardia and elevated liver enzymes were the only complications seen with greater frequency in the hypothermia group. Both have been shown to reduce mortality and improve neurological outcome following moderate H-I injury but only one recent whole body hypothermia study claimed improved outcome following severe H-I injury (Shankaran et al., 2005). Hypothermia is effective only if started within 6 hours after injury or prior to onset of delayed seizures. In addition to the use of hypothermia, treatment of seizures is generally thought to be desirable. It should be remembered that in immature brain GABA receptors can have an excitatory function rather than an inhibitory function. Drugs such as topiramte, which block fast-sodium channels and AMPA/kainite receptors, may be advantageous over benzodiazepines. Topiramate combined with hypothermia provided improved functional and pathologic outcome not provided by either modality alone in a rodent model of neonatal H-I (Liu et al., 2004).

A wide variety of treatments following H-I injury have been tried in animals including NMDA receptor blockade, AMPA/kainite receptor blockade, infusion of neurotropic factors such as BDNF into cerebral ventricles (Almli et al., 2000) and erythropoietin (Kumral et al., 2004). All have reported improved outcomes following neonatal H-I in whole animal trials using behavioral endpoints. Other animal trials have combined therapies in an attempt to improve outcomes. Human trials lag the animal studies by years. At present human trials have been proposed for erythropoietin in Europe. Much work remains to be done in order to improve the outcome following H-I in newborns. At present clinical suspicion for risk and judicious intra-operative care are the best means of avoiding exacerbation of a pre-existing condition.
REFERENCES:


