Published in final edited form as:
Clin Perinatol. 2009 December ; 36(4): 859–880. doi:10.1016/j.clp.2009.07.013.

Neuroprotection in the Newborn Infant

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Synopsis

Neonatal brain injury is an important cause of death and disability, with pathways of oxidant stress, inflammation, and excitotoxicity that lead to damage that progresses over a long period of time. Therapies have classically targeted individual pathways during early phases of injury, but more recent therapies such as growth factors may also enhance cell proliferation, differentiation and migration over time. More recent evidence suggests combined therapy may optimize repair, decreasing cell injury while increasing newly born cells.

Keywords

neonatal stroke; hypoxia; ischemia; neuroprotection; neurogenesis

Causes of early brain injury include stroke, birth trauma, metabolic or genetic disorders, status epilepticus, and asphyxial events. Perinatal asphyxia presents as encephalopathy, or hypoxic ischemic encephalopathy, occurring in 3 to 5 in 1000 live births [1], while stroke studies conservatively estimate an incidence of 1 in 4000 live births [2]. It is classically thought that hypoxic-ischemic (HI) injury leads to periventricular white matter damage in premature infants, while term infants develop cortical/subcortical lesions [3], but more recent evidence suggests that this distinction in injury type may not be so clear [4]. While many suffering from perinatal brain injury die during early life, the majority of survivors exhibit neurological deficits that persist, such as cerebral palsy, mental retardation or epilepsy [5]. Aside from hypothermia, no established therapies exist, and treatment and care for the sequelae of early brain injury requires significant resources. Even after maximal care, there is often little improvement in an individual's overall abilities, with long-term effects on the family, health care system, and society [6].

A search for therapies that can prevent injury progression or enhance repair of the immature brain continues, with the goal of improving long-term motor and cognitive outcomes. Because the neonatal and adult brain do not respond to insults in the same manner, secondary to differences in gene regulation during hypoxia and altered susceptibility to oxidative stress and excitotoxicity, alternate therapies must be sought [7]. Damage occurs via multiple pathways,

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and repair occurs over a period of days to weeks, if not months [8]. While some therapies that manipulate injury pathways show promise, not all neonates will benefit from treatment. Damage may be so severe or prolonged that repair may not be possible, or survivors may be particularly devastated [9].

The term “neuroprotection” is frequently used to describe the treatment response to brain injury, but should we think only about protecting neurons? Optimizing therapy for early brain injury requires capitalizing on multiple pathways that not only prevent cell death, but also enhance cell growth, differentiation, and long term integration into neural networks. In addition to neuronal damage, injury to non-neuronal cell types, such as oligodendrocytes and astrocytes, adversely affects development and results in long-term morbidity. By targeting the response to injury, the goal is to utilize selected pharmacotherapies to salvage cells that would otherwise die, protect cells from becoming injured or at risk for death by increasing tolerance, and also repair injured cells and enhance neurogenesis. Recent evidence suggests that therapies may be combined to enhance the protective and reparative processes, and thought must be given to the best time to administer these interventions. Clearly, because injury evolves over long periods of time with different mechanistic phases, therapies will also need to be administered over long periods of time, with different drugs aimed at these temporally evolving targets.

To maximize the efficacy of post-injury treatment, we need to identify quickly those neonates that will benefit from these therapies. A variety of clinical predictors have been used to identify those at risk for hypoxic brain injury. These include low Apgar scores, cord blood or early arterial acidosis, and seizures or the presence of encephalopathy on examination [10]. Cerebral function monitoring using bedside amplitude integrated EEG (aEEG) has provided an efficient means for identifying encephalopathy or prolonged seizure [11], but it does not replace full EEG [12]. Brain imaging with magnetic resonance imaging (MRI), including newer techniques such as spectroscopy (MRS), diffusion weighted (DWI) and diffusion tensor imaging (DTI), and volumetric analyses, provides the most accurate assessment of injury [13]. These techniques allow determination of the severity and evolution of brain injury, with specific injury patterns being associated with poor outcomes such as loss of gray/white differentiation, watershed injury, and thalamic or basal ganglia injury [14]. However, early and sequential imaging in neonates is often not possible because of scanner availability or difficulty in transporting these critically ill patients. Biomarkers for oxidative stress and inflammation, or indicators of injury to other organ systems, are currently being studied but are of equivocal value in identifying early neonatal brain injury. Given all of the available evidence, a combination of encephalopathic physical exam and seizures provides the best estimate of infants that may be at risk for brain injury [10]. This review will focus on recent developments in treating neonatal brain injury, as well as on combination therapy that will potentially enhance repair and optimize long-term outcomes.

HYPOTHERMIA

Therapeutic hypothermia has now become standard of care for neonatal HI brain injury. Multiple animal models of perinatal brain injury demonstrate histological and functional benefit of early initiation of hypothermia [15-19] (Table 1). Brief hypothermia provides partial neuroprotection [20,21], but prolonged moderate hypothermia to 32-34°C for 24-72 hours results in sustained improvement in behavioral performance in both newborn and adult animals [18,19]. The only complications noted are transient effects on heart rate and blood pressure [22].

Studies of therapeutic hypothermia in human neonates show a reduction in mortality and long-term neurodevelopmental disability at 12-24 months of age, with the most benefit seen in moderately encephalopathic infants [9,23-25]. Sustained protection does depend on the dose

of hypothermia, with maximum benefit obtained with cooling to 33-34°C, as well as on limited delay to treatment initiation [18,26]. Mild hypothermia to this level appears to be well tolerated without serious adverse effects if initiated within the first 6 hours of life [23,27-29]. Recent evidence shows that there are no changes in arterial blood pressure [30], but there may be some mild changes in blood gas parameters [31]. There also appears to be an increased risk of pulmonary hypertension in cooled infants, although generally not severe [32]. In selective head cooling, treatment benefits infants with moderate, but not severe, aEEG changes, improving survival without severe neurodevelopmental deficits or an increase in complications [9]. In addition to severity of encephalopathy, larger infants appear to be more responsive to hypothermia and at more risk for injury if hyperthermic at any point [33,34]. In a second multicenter trial, whole-body cooling to 33.5°C initiated within 6 hours and continued for 72 hours resulted in less death and severe disability at 18 to 22 months [35]. Whole-body cooling may be more effective in reducing temperature in the deep brain structures [36], and may be more feasible in certain clinical settings [37].

GROWTH FACTORS

The response of the immature brain to milder forms of injury can help us learn about mechanisms the brain uses to protect itself from insults. Animals treated with sublethal stress are protected from subsequent insults that would otherwise be deadly [38,39]. For example, immature rats that are exposed to hypoxia have reduced brain injury following HI that occurs 24 hours after this preconditioning stimulus, with protection that persists 1-3 weeks later [40, 41]. It is possible that injury may only be delayed, and protection may not be permanent; however, hypoxic preconditioning does provide long-lasting histological and functional protection for up to 8 weeks after neonatal rodent HI [42].

Hypoxia-inducible factor 1α (HIF-1α) activation is a key modulator of the protection against subsequent HI injury that is induced by hypoxic preconditioning [38,43]. HIF-1α is a neuronal transcription factor that stabilizes during hypoxia by binding to HIF-1β. Following stabilization, it produces a variety of downstream targets that are neuroprotective, including insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and erythropoietin (EPO).

EPO is a 34-kDa glycoprotein that was originally identified for its role in erythropoiesis, but has since been found to have a variety of other roles. Functions include modulation of the inflammatory and immune responses [44], vasogenic and proangiogenic effects through its interaction with VEGF [45,46], as well as effects on central nervous system (CNS) development and repair. EPO and EPO receptor are expressed by a variety of different cell types in the CNS, with changing patterns during development [47]. EPO plays a vital role in neural differentiation and neurogenesis early in development, promoting neurogenesis in vitro and in vivo [48].

Increasing evidence suggests that exogenously administered EPO has a protective effect in a variety of different models of brain injury. Post-injury treatment protocols in newborn rodents have demonstrated both short- and long-term histological and behavioral improvement [49]. A single dose of EPO given immediately after neonatal HI injury in rats significantly reduces infarct volume and improves long-term spatial memory [50]. Single- and multiple-dose treatment regimens of EPO following neonatal focal ischemic stroke in rats reduce infarct volume [51] and improve both short-term sensorimotor [52] and long-term cognitive [53] outcomes, but there may be more long-lasting behavioral benefit in female rats [54]. EPO treatment initiated 24 hours after neonatal HI also decreases brain injury [55]. In addition, EPO enhances neurogenesis and directs multipotential neural stem cells toward a neuronal cell fate [45,48,56]. Following transient ischemic stroke, there is a temporary precursor-cell

proliferation in the rodent subventricular zone (SVZ), a source of endogenous precursor cells throughout the life of the rodent, with this precursor-cell proliferation and differentiation favoring gliogenesis [57]. EPO has been shown to enhance neurogenesis in vivo in the SVZ following stroke in the adult rat [45]. Neurogenesis has also been demonstrated following EPO treatment, with an increase in newly generated cells from precursors [45,48,58] and possibly also an effect on cell fate commitment in vitro [45,48].

In humans, EPO is safely used for treatment of anemia in premature infants [59]. EPO for neuroprotection is given in much higher doses (1000-5000 U/kg/dose) than for anemia, to enable crossing of the blood-brain barrier [52,60,61], with unknown pharmacokinetics in humans. Recently, extremely low birthweight infants tolerated doses between 500 and 2500 U/kg/dose [62] (Table 2), and studies are ongoing.

VEGF is a regulator of angiogenesis that is also involved in neuronal cell proliferation and migration [63]. The endothelial microenvironment establishes a vascular niche that promotes survival and proliferation of progenitor cells, events which are tightly coordinated with angiogenesis [64]. VEGF-A is the most important member of a family of growth factors that also includes placental growth factor (PLGF) and VEGFs B, C, and D. VEGF-A is expressed in cortical neurons during early development, switching to mature glial cells near vessels during maturation. Following exposure to hypoxia, there is increased neuronal and glial expression of VEGF-A [65], directing vascularization and stimulating proliferation of neuronal and non-neuronal cell types [66-68]. VEGF also has chemotactic effects on neurogenic zones in the brain [69], increasing migration of stem cells during anoxia [70,71]. VEGF knockout mice have severe impairments in vascularization, neuronal migration and survival [72].

In adult ischemia models, intravenous VEGF administered 1 hour after insult increases blood-brain barrier leakage and lesion size, but late administration 48 hours after ischemia enhances angiogenesis and functional performance [73]. Both topical and intracerebroventricular injection reduced infarct volume [74,75], and benefit has been shown in neurodegenerative and traumatic models of injury as well. VEGF-overexpressing mice also show benefit from direct neuroprotection resulting from inhibition of apoptotic pathways [63].

Other trophic factors have also shown promise, but given their role in normal neurodevelopment the effects of treatment are not known. IGF-1 is important for growth and maturation of the fetal brain as well as differentiation of oligodendrocyte precursors [76]. IGF-1 has prosurvival properties that can prevent perinatal hypoxic and excitotoxic injury [77,78], and is also effective after intranasal administration [79]. Brain-derived neurotrophic factor (BDNF) is a neurotrophin that also provides neuroprotection in neonatal HI [80-83]. BDNF prevents spatial learning and memory impairments after injury, but its effectiveness is limited by the stage of development [82,83]. While protective in mice when given on postnatal day 5 (P5), BDNF has no effect at later time points and actually exacerbates excitotoxicity if given on the day of birth [82].

STEM CELL THERAPY

Neural stem cells (NSCs) are multi-potent precursors that self renew and retain the ability to differentiate into a variety of neuronal and non-neuronal cell types in the CNS. They reside in neurogenic zones throughout life, such as the SVZ and the dentate gyrus of the hippocampus in rodents, and are responsible for maintaining baseline turnover of cells as well as replacing injured cells through migration to penumbral tissue after injury. NSC transplantation has shown potential as a therapeutic strategy in adult animal models of brain injury. Implanted cells integrate into injured tissue [84], decreasing volume loss [85-87] and improving behavioral outcomes [88,89]. In neonatal models, intraventricular implantation of NSCs after HI results in their migration to injured areas [86,87] and differentiation into neurons, astrocytes,

oligodendrocytes, and undifferentiated progenitors. These cells promote regeneration, angiogenesis and neuronal cell survival in both rodent and primate models, and non-neuronal progeny inhibit inflammation and scar formation [90,91]. While complications of implantation have not been noted in these models, efficacy does depend on time of implantation, and the therapeutic window is not known. More recent technology enables labeling of stem cells, which can then be tracked from the site of implantation through their migratory path into the ischemic tissue [92-95], making their identification and eventual outcome in humans possible.

ANTIOXIDANTS

Oxidative stress is an important component of early injury to the neonatal brain [96], resulting from the excess formation of free radicals (FR) [reactive oxygen species (ROS) and reactive nitrogen species (RNS)] under pathological conditions. These include superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2^{\cdot}$) and hydrogen peroxide (H_2O_2) [97,98]. Antioxidant defenses such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, and compounds such as vitamins A, C, E, beta carotene, glutathione and ubiquinones scavenge FRs under normal conditions. Damage occurs when there is an imbalance between their generation and uptake [97]. Following HI, there is an increase in superoxide and hydroxyl radical production and rapid depletion of antioxidant stores, which leads to cell membrane damage, excitotoxic energy depletion, cytosolic calcium accumulation, and activation of pro-apoptotic genes that cause damage to cellular components and result in cell death [99].

The neonatal brain has a high rate of oxygen consumption and low concentration of anti-oxidants, making it susceptible to damage [100,101]. In the rat, total GPx activity increases between embryonic day 18 (E18) and postnatal day 1 (P1), but is still at lower levels than that seen in the mature brain [102]. In humans, mature oligodendrocytes carry increased antioxidant enzymes compared with the oligodendrocyte precursors present in the immature brain, which may partially explain the susceptibility of premature infants to white matter damage [103-105].

In an effort to reduce oxidative damage to the neonate, a number of strategies have been employed including ROS scavengers, lipid peroxidation inhibitors, FR reducers, and nitric oxide synthase inhibitors. Nitric oxide synthase (NOS) catalyzes the synthesis of nitric oxide (NO) from the conversion of arginine to citrulline [106]. NO plays an important role in pulmonary, systemic, and cerebral vasodilation, and is constitutively produced in response to increased intracellular calcium by endothelial nitric oxide synthase (eNOS) in endothelial cells and by neuronal nitric oxide synthase (nNOS) in astrocytes and neurons. An inducible isoform of nitric oxide synthase (iNOS) also produces NO in response to cellular stress, which initiates neuronal damage when converted to secondary reactive nitrogen species that facilitate nitration and nitrosylation reactions [107]. Early endothelial NO is protective by maintaining blood flow, but early neuronal NO and late inducible NO promote cell death [108]. Brain iNOS is induced in multiple cell types during upregulation of the pro-inflammatory pathway after brain injury [109], modifying binding to NMDA receptors and enhancing excitotoxicity [110].

Selective inhibition of nNOS or iNOS has shown potential as a neuroprotective strategy [111]. Regions expressing nNOS correspond to those that are susceptible to excitoxicity, expressing NMDA receptors in vivo and in vitro [112-114]. Destruction of neurons containing nNOS or targeted disruption of the nNOS gene protects animals from HI injury [115] [113], but nonspecific blockade of nNOS and eNOS is not protective [116]. There have been few studies in human newborns examining cerebral NO production. Cerebrospinal fluid (CSF) NO levels increase with severity of HI encephalopathy at 24 to 72 hours after asphyxia [117], with increased NO and nitrotyrosine levels in the spinal cord as well [118]. Initial results in premature infants treated with inhaled NO for prevention of bronchopulmonary dysplasia show

reductions in ultrasound-diagnosed brain injury and improvements in neurodevelopmental outcomes at 2 years of age, but long-term results are still pending [119,120].

Several other antioxidant strategies that either block FR production or increase antioxidant defenses are being studied. Melatonin is an indoleamine that is formed in higher quantities in adults and functions as a direct scavenger of ROS and NO. It has been found to provide long-lasting neuroprotection in experimental HI and focal cerebral ischemic injury [121,122], and human neonates treated with melatonin were also found to have decreased pro-inflammatory cytokines [123,124]. Allopurinol has mixed effects that have shown promise in animal and human studies. Xanthine oxidase-derived superoxide and H_2O_2 react with NO to form damaging RNS. Allopurinol reduces FR production by inhibiting xanthine oxidase while also scavenging hydroxyl radicals. High-dose allopurinol given 15 minutes after HI in P7 rats decreases acute edema and long-term infarct volume [125]. Short-term benefits have also been seen in neonates undergoing cardiac surgery for hypoplastic left heart syndrome [126]. Early allopurinol in asphyxiated infants improved short-term neurodevelopmental outcomes and decreased serum NO levels after administration; however, there may be only a brief window for benefit, as no improvement in long-term outcomes was seen with later treatment after birth asphyxia [127]. Deferoxamine (DFO) is an iron chelator that decreases FR production by binding with iron and decreasing the production of $OH^{\cdot}$ that occurs via the Fenton reaction [128,129], while also stabilizing HIF-1α to produce its downstream products VEGF and EPO [128]. DFO is protective during exposure to H_2O_2 or excitotoxicity in vitro [130], and in animal models of HI and transient ischemic stroke in vivo [128,131,132]. N-acetylcysteine (NAC) is a glutathione precursor and FR scavenger that attenuates lipopolysaccharide-induced white matter injury in newborn rats [133,134], but results for other antioxidant compounds, such as vitamin E, have been inconclusive [135].

EXCITOTOXICITY

Glutamate plays an important role in progenitor cell proliferation, differentiation, migration and survival in the developing brain. Excitotoxicity refers to excessive glutamatergic activation that leads to cell injury and death [136]. Glutamate accumulates in the brain after HI [137] from a variety of causes, including vesicular release [138] and reversal of glutamate transporters [139,140]. Glutamatergic receptors include N-methyl-D-aspartate (NMDA), alpha-3-amino-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate. NMDA receptor activation, while important for synaptic plasticity [141], can increase intracellulular calcium and pro-apoptotic pathways via caspase-3 activation if overactivated [142,143]. Excitotoxicity has long been known to play a part in the progression of HI brain injury, and differences in receptor expression contribute to the vulnerability of the developing brain [144]. NMDA, as well as AMPA and kainate, receptors on oligodendrocyte precursors play a large part in their susceptibility to damage in premature HI-induced white matter injury [145-147].

There has long been a search for agents that decrease brain injury by decreasing excitotoxicity. Dizocilipine (MK801) is a noncompetitive NMDA receptor antagonist that has been studied in humans, but is poorly tolerated and has also been shown to increase apoptosis and decrease neuronal migration in animal models [148]. Memantine is a low affinity noncompetitive NMDA receptor antagonist that is well tolerated in adults for Alzheimer's-type dementia [149]. Post-HI treatment with memantine attenuates acute white matter injury in P6 rats, resulting in long-term histological improvement in vivo and restoring neuronal migration in vitro [150-152]. Another method to decrease excitotoxicity is the use of topiramate, an AMPA-kainate receptor antagonist that is an FDA-approved anti-epileptic for patients greater than 2 years of age. It has been shown to protect newborn rodents from excitotoxic brain lesions [153], reducing brain damage and cognitive impairment when administered within two hours

of the insult [154]. An IV preparation of topiramate does not yet exist for human use, but this treatment shows potential as a therapy for early newborn seizure and injury. Cannabinoids have also shown promise as a treatment for neurodegenerative disorders [155] and in adult models of ischemia [156] or trauma [157]. They are involved in control of synaptic transmission, and their receptors (CB1 and CB2) are expressed on neurons and glia [158,159]. In the immature brain, cannabinoids have effects on excitotoxic lesions [160], and the agonist WIN 55,212-2 reduces short-term brain injury when administered after neonatal HI [161].

Magnesium sulfate has shown some benefit in preventing white matter damage in animal models [162-164], and one possible mechanism of its neuroprotection is the blockade of NMDA receptors [165]. In a multicenter clinical trial of mothers treated with magnesium who were at risk for preterm delivery, no perinatal side effects were seen and there was some benefit in the neurodevelopment of survivors [166]. However, magnesium administered to asphyxiated term neonates did not result in improvements in aEEG patterns, and when given in larger doses was associated with profound hypotension [167,168].

ANTI-INFLAMMATORY THERAPY

Maternal infection is a known risk factor for white matter damage and poor outcomes, such as cerebral palsy [169-171]. The inflammatory response and cytokine production that accompanies infection may play a large role in cell damage and loss [172]. Local microglia are activated early and produce pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, as well as glutamate, FRs, and NO. Systemic administration of these cytokines increases excitotoxic lesions [173], while therapies that block microglial activation and cytokine release protect the brain from excitotoxic damage [174].

Minocycline is a tetracycline derivative that crosses the blood-brain barrier and has anti-inflammatory effects, including decreasing microglial activation and caspase-3 expression [175,176], lipid peroxidation [177], and other pro-inflammatory activity [178] while increasing anti-apoptotic gene expression [179,180]. Minocycline has shown promise in a number of animal models of neurodegenerative or ischemic disease [175,181-185]. In the neonatal brain, minocycline appears to decrease tissue damage and caspase-3 activation in rodents when given immediately before or after injury, but results are inconsistent [186-188]. Low- and high-dose regimens were effective in reducing short-term HI-induced inflammation, protecting developing oligodendrocytes [188] and myelin content in neonatal rats [189], but this effect was only transient in another study of neonatal rodent stroke [187]. Delayed therapy was found to decrease TNF-α and matrix metalloproteinase MMP-12, but efficacy was lost when treatment was extended for a week after stroke [190]. These effects also appear to be species dependent, with an increase in injury in developing C57B1/6 mice [191].

CELL DEATH INHIBITORS

Apoptosis is a critical component of normal brain development. While necrosis plays a major role in early neuronal death in both the immature and mature brain following injury [192], a spectrum of cell death that includes apoptosis occurs within the first 24 hours following neonatal HI [193], and may result in heterogeneous responses to anti-apoptotic therapies [194]. It is also probable that apoptosis and cleavage and activation of caspase-3 are responsible for more of the cell death that occurs in delayed phases of injury and neurodegeneration [195].

Specific and non-specific inhibition of caspases or cysteine proteases, which are highly activated after HI, has been attempted with some success [196-199]. For example, calpain or caspase-3 inhibitors such as MDL 28710 and M826 protect neonatal rats after HI [197,200]. Pretreatment with the hormone 17β-estradiol is neuroprotective in immature rats, and appears

to work through both anti-apoptotic and FR scavenging pathways [201]. In addition, the nuclear enzyme poly (ADP-ribose) polymerase (PARP) is activated during stress and enables DNA repair; however, the PARP-1 isoform also contributes to ischemic neuronal injury by depleting energy stores and activating microglia, leading to cell death. PARP-1 is more abundant in the immature brain, and its blockade protects against excitotoxicity and ischemic injury [202]. The PARP-1 inhibitor 3-aminobenzamide reduces injury after focal ischemia in P7 rats [203], but PARP-1 blockade appears to protect males preferentially [202].

COMBINATION THERAPY

Single therapy that attacks any of the aforementioned injury pathways often results in only mild improvement. For example, therapeutics targeting apoptosis may prevent delayed cell death, but would not effect earlier necrotic and excitotoxic injury. Hypothermia has become the standard of care in many institutions since showing benefit in moderately encephalopathic newborns; however, it does not completely protect or repair an injured brain, and benefits may not necessarily be long lasting [204,205], so the search for adjuvant therapies continues. Combinatorial therapy may provide more long-lasting neuroprotection, salvaging the brain from severe injury and deficits while also enhancing repair and regeneration, hopefully providing additive, if not synergistic, protection.

Xenon is approved for use as a general anesthetic in Europe and has shown promise as a protective agent. It is an NMDA antagonist, preventing progression of excitotoxic damage. It appears to be superior to other NMDA antagonists, possibly through inhibition of AMPA and kainate receptors, reduction of neurotransmitter release, or effects on other ion channels [206-208]. Combination xenon and hypothermia initiated 4 hours after neonatal HI provided synergistic histological and functional protection when evaluated at 30 days after injury [209]. Hypothermia does reduce glutamate and glycine release [210], and NMDA receptor antagonism may explain these effects. More recently, an additive effect was shown after HI in P7 rats that were cooled to 32°C and received 50% xenon, with improvement in long-term histology and functional performance that exceeded the individual benefit of either [211]. More extensive studies on xenon use in human neonates are necessary.

N-acetylcysteine (NAC) is a medication approved for neonates that is a scavenger of oxygen radicals and restores intracellular glutathione levels, attenuating reperfusion injury and decreasing inflammation and NO production in adult models of stroke [212,213]. Adding NAC therapy to systemic hypothermia reduced brain volume loss at both 2 and 4 weeks after neonatal rodent HI, with increased myelin expression and improved reflexes [214]. Inhibition of inflammation with MK-801 has also been effective when combined with hypothermia in neonatal rats post HI injury [215]. In P7 rats who underwent HI followed by early topiramate and delayed hypothermia, improved short-term histology and function was seen [216]. The inhibition of inflammation may provide a window for protection if hypothermia is delayed, which is possible given difficulty in initiation of cooling if infants are born at an outside hospital or transport is delayed.

CONCLUSION

Most studies have focused on singular mechanisms of injury, such as oxidative stress, inflammation, and excitotoxicity. More recent evidence suggests that injury occurs over long periods of time and that therapies may need to be administered over much longer periods than have been previously entertained. While hypothermia and single pharmacotherapies show promise, combined therapy may be necessary to increase the therapeutic time window for protection and repair, making recovery possible.

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Table 1

Hypothermia Studies (discussed in text)

Study	Species	Type of cooling	Primary outcome
Laptook, et al., 1994.	pig	whole body	Decreased histological/behavioral impairment
Towfigh, et al., 1994.	rat	head	Improved histology
Thoresen, et al., 1995	pig	whole body	Improved MRI measures
Gunn, et al., 1997.	ovine	head	Improved EEG/histology
Laptook, et al., 1997	pig	whole body	Less encephalopathy/histological damage
Bona, et al., 1998	rat	whole body	Improved histology/no change in sensorimotor function
Gunn, et al., 1998	human	head	Safe for mild systemic/moderate head cooling
Azzopardi, et al., 2000	human	whole body	Only mild abnormalities in VS seen with cooling
Thoresen & Whitelaw, 2000	human	head	Non-hazardous changes in HR/BP with cooling
Eicher, et al., 2005	human	whole body	Mild-mod abnormalities with cooling, improved outcomes
Gluckman, et al., 2005	human	head	Beneficial in infants with less severe EEG changes
Shankaran, et al., 2005	human	head	Reduced death/disability, trend toward improvement at 18-22 months
Wyatt, et al., 2007	human	head	Less death/disability at 18 months
Battin, et al., 2009	human	whole body	No effect on mean arterial blood pressure
Sarkar, et al., 2009	human	both	Pulmonary dysfunction common but not severe
Robertson, et al., 2009	human	whole body	Demonstrates ability to cool in low resource setting

BP=blood pressure; EEG=electroencephalogram; HR=heart rate; mod=moderate; MRI=magnetic resonance imaging; VS=vital signs

Table 2

Human Studies of Neuroprotectants (discussed in text)

Treatment	Mechanism	Study	Primary outcome
Hypothermia	multiple	(see Table 1)	
EPO	growth factor	Juul, et al., 2008	High dose EPO safe in ELBW infants
Inhaled Nitric Oxide	antioxidant	Schreiber, et al., 2003	Lower incidence of severe IVH and PVL
		Ballard, et al., 2006	Decreased neurodevelopmental disability
Melatonin	antioxidant	Gitto, et al., 2004	Decreased pro-inflammatory markers, nitrates/nitrites
		Gitto, et al., 2005	Reduced pro-inflammatory cytokines, improved clinical outcome
Allopurinol	antioxidant	Clancy, et al., 2001	Neurocardiac protection in HLHS infants
		Benders, et al., 2006	Postnatal treatment had no effect
Magnesium Sulfate	↓ excitotoxicity	*Levene, et al., 2002*	Increased Mg dose associated with hypotension
		Groenendaal, et al., 2002	No positive effect on aEEG patterns
		Crowther, et al., 2003	May improve pediatric outcomes when given to mothers during pregnancy
		Khashaba, et al., 2006	No effect on post-natal level of EAA

aEEG=amplitude integrated EEG; EAA=excitatory amino acids; ELBW=extremely low birth weight; HLHS=hypoplastic left heart syndrome; IVH=intraventricular hemorrhage; Mg=magnesium; PVL=periventricular leukomalacia