Hypoxic-Ischemic Brain Injury: Potential Therapeutic Interventions for the Future

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Educational Gaps
1. Further understanding of the pathophysiological mechanisms leading to neuronal death after hypoxia-ischemia will help guide therapeutic approaches.
2. As hypothermia is the only current treatment shown to reduce death or disability after hypoxia-ischemia, new therapeutic approaches require further investigation.

Abstract
Perinatal hypoxic-ischemic (HI) brain injury is a common problem with potentially devastating impact on neurodevelopmental outcomes. Although therapeutic hypothermia, the first available treatment for this disease, reduces the risk of death or major neurodevelopmental disability, the risk of major neurologic morbidity after HI remains significant. Basic research has identified cellular mechanisms that mediate neuronal death. This article reviews the cellular processes induced that lead to brain injury after HI, and identifies treatments currently under investigation for potential translation to clinical trials.

Objectives After reading this article, readers should be able to:
• Be familiar with the presentation of hypoxic-ischemic (HI) encephalopathy.
• Understand the primary mechanisms leading to neuronal injury after perinatal hypoxia-ischemia.
• Appreciate the variety of new approaches currently under investigation for treatment of newborns with HI brain injury.

Introduction
Perinatal hypoxic-ischemic (HI) brain injury is an important clinical problem in the neonate, leading to cerebral palsy and developmental delay, and affecting 1 out of every 1,000 live term births in the United States. (1) Brain hypothermia, induced by externally cooling either the head or the whole body in the first 6 hours after perinatal HI, is the only treatment currently employed to reduce death and disability, and only in term infants. (2)(3) Cooling decreases the incidence of the combined outcome measure of death or disability at 18 months to 2 years after HI. (2)(4)(5)(6) Disappointingly, however, long-term follow-up studies of neurodevelopmental outcomes in survivors from 2 large trials (2)(5) have not revealed cooled infants to have lower incidences of moderate or severe disability at school age compared with those who had not been cooled. (7)(8) These studies highlight the need for new therapies to rescue injured neurons after HI and improve neurodevelopmental outcomes. Current research suggests that achieving these goals may be possible with new approaches to treatment. To put these approaches in context, we will first briefly review the outcomes and presentation of HI brain injury.

Abbreviations
eNOS: endothelial nitric oxide synthase
EPO: erythropoietin
H2O2: hydrogen peroxide
HIE: hypoxic-ischemic encephalopathy
HI: hypoxic-ischemic
IL: interleukin
NMDA: N-methyl-D-aspartate
nNOS: neuron-specific nitric oxide synthase
NO: nitric oxide
O2−: superoxide
r-EPO: recombinant erythropoietin

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Presentation of Hypoxic-Ischemic Brain Injury

Encephalopathy (the neurologic syndrome comprising abnormalities of consciousness, tone, and autonomic control) is the hallmark of acute HI brain injury in the newborn. (9) In the absence of clinically obvious encephalopathy over the first 24 hours after birth, subsequent development of abnormal neurodevelopmental outcomes cannot be ascribed to perinatal hypoxia-ischemia. The stage of encephalopathy depends on the timing and severity of the HI, as well as the genetic endowment of the infant, so that encephalopathy severity can differ widely between infants who have experienced apparently similar insults. The relationship between clinical presentation and HI severity has been encapsulated in the commonly used (10)(11) Sarnat staging system. (9) Thus, infants with stage I encephalopathy demonstrate hyperalertness, increased sympathetic autonomic outflow, and normal to increased tone. Infants exhibiting stage II encephalopathy can present with a variety of consciousness levels, ranging from lethargy to obtundation, accompanied by increased parasympathetic autonomic function, mild hypotonia, and, commonly, seizures. Finally, infants with stage III encephalopathy exhibit stupor, flaccid tone, and depressed sympathetic and parasympathetic function. (9) Importantly, the sequelae of HI evolve over time: in stage I and II encephalopathies, end-organ injury resolves and consciousness improves; with stage III encephalopathy, infants can die, progress to a chronic vegetative state, or survive with severe impairments. Understanding this natural history can be helpful in determining when an intrauterine period of HI has taken place: an infant experiencing HI shortly before birth will demonstrate acute encephalopathy. In contrast, an infant who has experienced HI days before birth will exhibit signs consistent with the evolving sequelae of HI.

Cellular Mechanisms of Neuronal Death After Hypoxia-Ischemia

Neurons require a continuous supply of metabolic substrates, particularly glucose and oxygen. HI brain injury arises from the inter- and intracellular processes during and after an imbalance of availability and consumption of these substrates in the brain. In animal models, neuronal death after HI occurs in 2 phases (12): immediately after HI, neurons begin to die rapidly, likely because of necrosis, a process of cell death characterized by acute loss of plasma membrane integrity and loss of adenosine triphosphate. (13) In the second phase, neurons die over hours to days, (14) primarily via apoptosis, (15) an active, tightly regulated cascade of intracellular events. In human infants, neuropathologic evidence of classic neuronal apoptosis after HI is less clear than in animal models. (16) However, it is unquestionable that immediate and delayed neuronal death underlie the neurologic injury after HI in infants. The comparatively long duration of the second phase of HI-induced neuronal death, and its persistence after birth suggest that the severity and extent of this delayed death is more likely to be modified by therapy than is the early, immediate phase.

New approaches to rescuing neurons after perinatal HI have leveraged current understanding of the mechanisms of HI-induced brain injury to specifically target central mechanisms of neuronal death. To place these treatments in the context of the cellular mechanisms they target, we will briefly review the processes of excitotoxicity, free radical toxicity, and inflammation.

Excitotoxicity

Glutamate is a ubiquitous excitatory neurotransmitter in the brain. Under pathologic conditions, including HI, neuronal receptors for glutamate are over-activated due to a pathologically high concentration of glutamate in the extraneuronal space. This high concentration arises as a result of pathologically increased synaptic release of glutamate, dysfunction of glutamate uptake mechanisms, and release of glutamate from the intracellular metabolic pool. Glutamate receptor over-activation results in neuronal death, hence, excitotoxicity. Over-activation of the N-methyl-d-aspartate (NMDA) subtype of glutamate receptor has been highly implicated in neuronal death after HI. NMDA receptor over-activation allows intracellular calcium to rise to toxic levels and activates cytotoxic phospholipases, proteases, lipases, and endonucleases, leading to cell death. Calcium is also taken up by the mitochondria, leading to loss of adenosine triphosphate synthesis, oxidative stress, release of proapoptotic factors, and activation of the apoptotic cascade.

Free Radical Toxicity

Free radicals are molecules containing 1 or more unpaired electrons, which allow them to have increased intermolecular reactivity. The primary oxygen free radical generated in cells is superoxide anion (O$_2^-$). Superoxide is an important intracellular signaling molecule, as is its metabolite, hydrogen peroxide (H$_2$O$_2$). Together with the highly reactive hydroxyl radical, O$_2^-$ and H$_2$O$_2$ are the main oxygen-derived free radicals in the cell. Oxidative stress refers to increased levels of these radicals. Oxidative stress contributes to neuronal death after HI, (17) by degrading cellular proteins and DNA.
In addition to oxidative stress, increased production of a nitrogen free radical, nitric oxide (NO), is a central mechanism of HI-induced neuronal death. (18) Increased NO production is mediated by a neuron-specific NO synthase (nNOS) activated by HI- (and excitotoxicity-) induced elevations of intracellular calcium concentrations. A second isoform of NO synthase, endothelial NOS (eNOS), controls vascular resistance in all organs including the brain. Preserving eNOS activity during and after experimental HI improves cerebral blood flow and neuronal survival, (19) so treatments aimed at reducing neuronal NO production must specifically target nNOS and preserve eNOS activity. In addition to its direct effects, NO interacts with $O_2^-$ to form the highly reactive and toxic radical, peroxynitrite. (20) Peroxynitrite-mediated peroxidation of lipid constituents of cellular membranes (21) and oxidative modification of mitochondrial proteins (22) are important mechanisms of neuronal injury. In particular, lipid peroxidation alters cellular membrane structure and function, inducing cellular necrosis or triggering apoptosis.

**Inflammation**

Improved outcomes in animal models of HI after inhibition of inflammation (23) demonstrate that inflammation is an important mechanism of HI-induced neuronal death. After HI, microglia are activated, (24) producing proinflammatory cytokines, eg, interleukin (IL)-1 and tumor necrosis factor-$\alpha$. In addition, microglia-derived chemokines acutely increase, (25) recruiting peripheral immune cells to the brain. HI activates the complement cascade within the brain. (26) Complement activation results in the formation of membrane attack complexes, which form pores within plasma membranes, leading to cell lysis. (27) Thus, after HI, a coordinated inflammatory response in the brain arises that makes a significant contribution to HI-induced neuronal death.

**New, Potential Treatments for Hypoxic-Ischemic Brain Injury**

With increasing understanding of the mechanisms of HI-induced neuronal death in the newborn, new approaches to neuroprotection have revealed promise in preclinical studies and early clinical trials (Figure). Below, we review some of the most promising approaches, at different stages of development, from early stage research to clinical studies, and Food and Drug Administration (FDA) approval. Because these treatments may address mechanisms different from those mediating hypothermia-mediated neuroprotection, these new therapies may also provide additive neuroprotection to that available from hypothermia treatment.

**Erythropoietin**

Erythropoietin (EPO) is an endogenous, hypoxia-induced glycoprotein produced in the kidney, first shown to regulate hematopoietic function via EPO-specific receptors. (28) Currently approved to increase erythropoiesis in anemia,
recombinant EPO (r-EPO) has also been demonstrated in animal studies of HI to be neuroprotective. (29)(30)(31) Activation of neuronal EPO receptors prevents HI-induced activation of NMDA receptors and increases expression of antiapoptotic proteins, potentially reducing excitotoxicity and decreasing apoptosis. (31)(32) EPO receptor activation also inhibits HI-induced increases of peroxynitrite (oxidative stress) and inflammatory cytokines, potentially reducing free radical toxicity and inflammation. (32) Of particular relevance for neonatal HI, EPO receptor expression is abundant in the developing mammalian brain. (33) Systemically administered r-EPO after HI has been shown to cross the blood-brain barrier (34); in 1 study, in infants who were given EPO after HI, the pharmacokinetics of EPO levels in cerebrospinal fluid paralleled that observed in serum, (35) suggesting that r-EPO may cross the blood-brain barrier in humans.

Clinical trials of r-EPO in infants after HI brain injury have begun. A phase 1, multicenter, open label, dose-escalation trial recently revealed that EPO is well-tolerated in term infants undergoing hypothermia as treatment for hypoxic-ischemic encephalopathy (HIE), without serious adverse effects at plasma concentrations shown to be neuroprotective in animals. (36) In the only prospective, randomized clinical trial to date, r-EPO reduced the risk of death or moderate/severe disability in term infants with HIE who were not treated with hypothermia. Notably, improved outcomes were restricted to infants in the moderate HIE subgroup. (35) However, the lack of blinding in this study raises concerns for its validity. Furthermore, because the patient population was restricted to patients who could pay for r-EPO, the generalizability of these results to other populations is unclear.

**Melatonin**

Melatonin is a pineal gland hormone secreted in response to environmental light-dark cycles. (37) Melatonin has multiple cellular effects, 2 of which directly target known mechanisms of HI brain injury. First, melatonin reduces free radical toxicity, scavenging hydroxyl radical and peroxynitrite by direct electron transfer. (38) Melatonin also decreases O$_2^-$ production in brain slices in vitro after HI stress. (39) Second, melatonin has anti-inflammatory activity. Thus, after umbilical cord occlusion in fetal sheep, melatonin decreased the production of 8-isoprostanes, (40) potent mediators of HI-induced inflammation. Furthermore, melatonin, given immediately to rats after focal cerebral ischemia, decreased the extent of neutrophil migration and macrophage-activated microglial infiltration 48 hours later, and only in the ischemic hemisphere. (41) Finally, melatonin reduces NF-κB binding to DNA, ultimately decreasing the production of proinflammatory cytokines, including IL-2, IL-6, and tumor necrosis factor-α. (42) These cellular effects have led to extensive investigation of melatonin as a treatment for HI brain injury.

In adult rats, melatonin, given after focal cerebral ischemia, improves short-term evaluations of infarct size and neurobehavioral outcomes, (41) suggesting that melatonin treatment may be applicable to global brain ischemia in the neonate. However, short-term improvements may reflect only transient inhibition of death-inducing processes without altering the ultimate extent of neuronal death. More encouragingly, melatonin provided to neonatal mice before and after severe hypoxia significantly increased hippocampal neuronal survival at 3, 7, and 14 days, as well as functional motor outcomes 2 weeks after insult. (43) Some data suggest that antenatal treatment with melatonin may be beneficial in improving outcomes from birth asphyxia: antenatal melatonin, provided to spiny mouse dams for 1 week before in utero global asphyxia of the fetuses, improved cortical neuronal survival at 24 hours after birth. (44) Finally, melatonin effects may be additive to the neuroprotective effects of induced hypothermia. After induction of global ischemia in neonatal pigs, melatonin combined with hypothermia decreased magnetic resonance spectroscopic indices of impaired cerebral energy metabolism compared with hypothermia alone. (45) Low levels of indices have high specificity in identifying asphyxiated infants who subsequently have normal neurodevelopmental outcomes at 1 year of age. (46) In the only study of melatonin and asphyxiated infants to date, melatonin given in the first 6 hours after birth decreased levels of malonaldehyde, a product of lipid peroxidation (47) in serum, the clinical importance of which is unknown. A randomized, double-blind placebo phase I study evaluating the effect of melatonin on infants undergoing hypothermia as treatment for HI brain injury is planned to begin in late 2013. (48)

**Allopurinol**

Allopurinol is an inhibitor of xanthine oxidase, a source of cytosolic O$_2^-$ during HI that has received interest as a potential neuroprotective agent, especially as it can cross the placenta to produce therapeutic levels in newborns. (49) Animal models including in vivo and in vitro rat models and in vivo sheep models have revealed allopurinol to be neuroprotective. (50)(51)(52)(53)

Neonatal trials after HI brain injury have been limited. One randomized, placebo-controlled trial enrolled 32 severely asphyxiated infants (overall mortality rate, 72%),
and revealed no outcome differences between the groups. (54) However, in a larger randomized study of 60 infants having a range of asphyxia severities, allopurinol treatment significantly decreased death or severe disability at 1 year of age. (55) Although this single study demonstrates some potential for postnatal allopurinol treatment of affected infants, interest is currently more focused on prenatal treatment, as reactive oxygen species are produced during HI in utero. During intrapartum asphyxia in fetal lambs, maternal administration of allopurinol suppressed superoxide production during intermittent partial umbilical occlusion (56) and decreased fetal hippocampal injury, (50) suggesting that providing allopurinol to fetuses at risk for HI may be helpful. In fact, in a randomized double-blind placebo-controlled study of 53 pregnant women whose fetuses demonstrated evidence of hypoxia, arterial cord blood from infants of allopurinol-treated mothers exhibited lower levels of S-100B, a marker of brain injury, a very short-term outcome. A randomized double-blind placebo-controlled trial of antenatal allopurinol treatment is ongoing with the goal of determining allopurinol effects on asphyxia-associated mortality and long-term neurodevelopmental outcome. (57)

**Topiramate**

Topiramate is a newer antiepileptic drug that has attracted interest as a potential neuroprotective agent for HI brain injury. Topiramate prevents seizures by inhibiting neuronal excitability, including through blockade of glutamate receptors. (58) This potentially anti-excitotoxicity effect suggests topiramate as a candidate therapy for HI brain injury. Indeed, after carotid artery ligation in rats, topiramate significantly reduced neuronal death through inhibition of glutamate receptor activity, (59) decreasing HI-induced neuronal apoptosis. (60) Of significant interest is the observation that topiramate has added neuroprotective effects in animal models when combined with hypothermia. (61)

In a pilot study, topiramate, given in conjunction with whole body hypothermia to 27 asphyxiated infants, caused no adverse effects, short-term outcomes differences, or incidence of pathologic brain magnetic resonance imaging compared with 27 controls. (62) Data from 2 phase I trials, one ongoing in the United States (63) and one recently completed in Italy (64) are awaited. Additional large clinical trials are needed to evaluate the efficacy of topiramate in preventing HI injury. Ultimately, the limitation of oral administration to critically ill infants may restrict the scope of use.

**Xenon**

Xenon is a chemically nonreactive gas that has undergone intensive investigation in Europe as a general anesthetic, (65)(66) due to its highly favorably safety profile. One of xenon’s activities is against NMDA receptor activation, decreasing excitotoxicity. This decreased activity arises from xenon block of glycine binding to its regulatory site on the receptor. (67) After hypoxia or excitotoxicity in cultured murine neurons, increasing concentrations of xenon significantly increased neuronal survival. (68) In neonatal rats, xenon inhalation improved both histologic and functional outcomes 2 months after global HI. (69) Similarly, after global forebrain ischemia in neonatal pigs, xenon inhalation markedly improved neuronal survival 72 hours after insult. (70) Notably, xenon-induced neuroprotection in these models has been found to be additive to the neuroprotection afforded by induced hypothermia.

In infants, preliminary evidence from a pilot study employing 12 asphyxiated infants indicates that adding xenon (50%) inhalation to ongoing hypothermia does not significantly alter blood pressure, heart rate, or FIO2 requirement. (71)(72) Currently, 2 phase I trials are ongoing in the United Kingdom, (73)(74) to further evaluate the safety and efficacy of xenon paired with cooling in infants with HIE. 

Even if xenon is determined in phase II to III trials to be effective in improving long-term neurodevelopmental outcomes after HI brain injury, the obstacles to its routine use for asphyxiated infants are significant, because its costs are very high, and because Xenon requires a closed circuit delivery system.

**nNOS Inhibition**

The central role played by NO in HI-induced neuronal injury and the availability of specific small molecule inhibitors of nNOS make nNOS inhibition a potentially attractive approach. With the discovery of the toxic role of NOS in HI, initial studies of NOS inhibitors produced conflicting results (75), due to the lack of isoform specificity of the early inhibitors. However, newer, specific nNOS inhibitors may have more promise. (71) In preterm fetal sheep, prophylactic use of the highly specific nNOS inhibitor, Jl-10, improved neuronal survival after profound asphyxia. (76) In addition, a second, highly selective nNOS inhibitor, Jl-8, improved locomotion scores and muscle tone in a rabbit model of intrauterine HI-induced cerebral palsy. (77) Although initial data for selective nNOS inhibitors are promising, the extent of off-target effects, such as inhibition of eNOS activity and any accompanying decrease in cerebral blood flow...
(19) will need to be explored before clinical trials can begin.

**Pluronic Copolymers**

After HI, the functions of cellular membranes can become altered, due to lipid peroxidation and alterations of lipid signaling. After severe HI, neuronal plasma membrane dysfunction leads to decreased membrane integrity, leakage of intracellular constituents into the extracellular space, and necrosis. When HI is not sufficiently severe to induce necrosis, HI-induced dysfunction of the intracellular membranes of mitochondria can trigger apoptosis. (78) Recently, a class of synthetic molecules, the pluronics, has been used to address HI-induced dysfunction of injured neuronal membranes in vitro and in vivo. Pluronics consist of chains of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) arranged in a tri-block, PEO-PPO-PEO, structure. This structure enables pluronics to interact with cellular membranes (79)(80), and to restore plasma membrane integrity after injury. One member of the pluronics, pluronic F-68, has been shown to profoundly rescue neurons from death in in vitro models of HI through blockade of apoptosis. (81)(82) Preliminary evidence also indicates that pluronic F-68, provided to animals for 1 week after HI, markedly improves neuronal survival in the hippocampus, a brain region highly vulnerable to global HI, and rescues hippocampus-mediated behavior. (83) The novelty of this membrane-targeted approach and its lack of toxicity (84)(85) suggest that targeting membrane dysfunction may constitute a viable treatment for HI brain injury in the future.

**Conclusions**

HI brain injury in the newborn arises from a simple imbalance between the brain’s demand for energy and its supply in the perinatal period. Yet the cellular mechanisms that lead to neuronal death are complex and multifactorial. The overall efficacy of induced hypothermia is relatively low, and the need for mechanism-directed treatments for HI brain injury is high. Basic research, in identifying the mechanisms underlying HI-induced neuronal death, can provide therapeutic targets for translational testing. The approaches discussed above target the cellular mechanisms of HI-induced neuronal death in vastly different ways (Figure). With continued research, 1 or more of these approaches, or derivatives of them, may ultimately become effective treatments for HI brain injury in the newborn.

**American Board of Pediatrics Neonatal-Perinatal Content Specifications**

- Know the incidence, causes, and pathophysiology, including cellular abnormalities, of acute perinatal asphyxia.
- Know the clinical features, diagnosis, and management of perinatal hypoxic-ischemic encephalopathy.
- Know the management of perinatal asphyxia, including neural protective strategies.

**References**


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1. A term boy infant is born to a mother who has placental abruption and there is a concern for hypoxic-ischemic (HI) brain injury. Which of the following characteristics and assessments about this patient would be correct?

   A. Even if the initial presentation in the first postnatal day appears normal without neurologic symptoms, encephalopathy can be attributed to a perinatal event such as abruption if the first symptoms occur before 72 hours after delivery.
   
   B. Hyperalertness and increased sympathetic tone without lethargy would be classified as stage II in the Sarnat system.
   
   C. The key to hypothermia treatment for patients such as this one is to start almost immediately after birth, as the treatment primarily targets the initial phase of sudden neuronal death, and has little to no effect on the second phase of apoptotic cell death.
   
   D. If this neonate has HI brain injury, there is likely to be an increased synaptic release of glutamate and over-activation of glutamate neuronal receptors.
   
   E. In HI injury primarily because of hypovolemia and anemia such as in placental abruption, there is decreased production of nitric oxide and subsequent persistent pulmonary hypertension associated with brain injury.

2. A newborn term girl infant is born after shoulder dystocia, has seizure activity 1 hour after birth, and is diagnosed with HI brain injury. She is enrolled in a trial of erythropoietin therapy. Which of the following is true regarding erythropoietin and its potential role in treatment for this condition?

   A. Because erythropoietin therapy for ischemic injury is primarily for conditions such as acute blood loss, it is not applicable for this clinical situation.
   
   B. The neuroprotective mechanism of erythropoietin may result from the prevention of N-methyl-D-aspartate receptor activation that can occur after HI injury.
   
   C. Although erythropoietin is well tolerated in preterm infants, a difficulty in its use in term infants has been its nephrotoxicity, which occurs in up to one-third of patients with HI injury.
   
   D. Recombinant erythropoietin does not cross the blood-brain barrier in most circumstances, but due to the capillary leak seen in HI injury, cerebrospinal fluid levels of erythropoietin reach approximately 25% of serum levels for such infants.
   
   E. Because erythropoietin receptors are not present in the neonatal brain, the mechanism of action is likely an indirect one that occurs primarily through renal erythropoietin receptors.

3. In a clinical trial for patients with HI encephalopathy, allopurinol is being tested. Which of the following is correct regarding allopurinol treatment?

   A. Allopurinol is an inhibitor of xanthine oxidase, which is a source of superoxide.
   
   B. The main use of allopurinol for this condition is for second-line or adjunctive treatment of neonatal seizures.
   
   C. Allopurinol is primarily being studied in the context of treatment for “missed” cases of HI injury, when diagnosis occurs after 6 hours of age.
   
   D. The main mechanism of allopurinol for HI injury is in the reconstruction of cell membranes that may have been damaged during the second phase of reperfusion injury.
   
   E. A key feature of allopurinol is its selective targeting of neuronal nitric oxide synthase, inhibiting nitric oxide production only in affected areas of brain injury.
4. Which of the following statements regarding melatonin and its potential role in the therapy for HI brain injury is correct?

   A. Melatonin is an artificial compound that has similarities to narcotics, and is primarily being considered as a sedative used for HI events, not having a direct effect on brain injury.
   B. Melatonin has been shown to reduce the extent of neutrophil emigration in animal models of HI brain injury.
   C. In human neonates, melatonin has been shown to lead to increased levels of malonaldehyde, a product of lipid peroxidation and a marker for neuronal regeneration.
   D. Melatonin administration soon after birth is associated with increased peroxide and superoxide levels in animal models.
   E. Melatonin administration is associated with increased levels of interleukin-2 and interleukin-6 levels, which may also help to prevent infections associated with HI injury.

5. Which of the following statements regarding potential new therapies for HI brain injury is correct?

   A. Xenon is a unique potential treatment for neonatal HI brain injury, being the only one that may have a role solely in the antenatal period.
   B. Allopurinol may be most useful in cases of HI injury that were diagnosed late, as its mechanism of action is primarily not prevention of injury, but reconstruction of injured neurons by facilitating cell nutrient uptake.
   C. Topiramate is an antiepileptic that may have a role in HI brain injury, inhibiting neuronal excitability by blocking glutamate receptors.
   D. Pluronic copolymers are a class of naturally occurring compounds that have been used primarily in applications to “sweep up” bacteria during sepsis, but may have a role in absorbing antioxidants such as peroxide and peroxynitrite.
   E. A major challenge in the use of pluronic copolymers is its nephrotoxicity and cardiotoxicity, with which has been observed in animal models and human adult trials.
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