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Review Article

Perinatal Hypoxic-Ischemic Encephalopathy

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Perinatal hypoxic-ischemic encephalopathy (HIE) is an important cause of brain injury in the newborn and can result in long-term devastating consequences. Perinatal hypoxia is a vital cause of long-term neurologic complications varying from mild behavioural deficits to severe seizure, mental retardation, and/or cerebral palsy in the newborn. In the mammalian developing brain, ongoing research into pathophysiological mechanism of neuronal injury and therapeutic strategy after perinatal hypoxia is still limited. With the advent of promising therapy of hypothermia in HIE, this paper reviews the pathophysiology of HIE and the future potential neuroprotective strategies for clinical potential for hypoxia sufferers.

1. Introduction

Perinatal hypoxic-ischemic encephalopathy (HIE) occurs in one to three per 1000 live full-term births [1]. Of affected newborns, 15%–20% of affected newborns will die in the postnatal period, and an additional 25% will develop severe and permanent neuropsychological sequelae, including mental retardation, visual motor or visual perceptive dysfunction, increased hyperactivity, cerebral palsy, and epilepsy [2]. The outcomes of HIE are devastating and permanent, making it a major burden for the patient, the family, and society. It is critical to identify and develop therapeutic strategies to reduce brain injury in newborns with HIE. The underlying pathophysiology of perinatal HIE is difficult to study in the human, thus the neonatal rat model for HI brain injury has been developed to model this human condition. Much of what we know is derived from studies conducted in animal models. Rodents were the most frequently used animals in HIE research, followed by piglets and sheep [3].

2. Gestational and Chronological Age

The neuropathological features of perinatal HIE vary considerably with the gestational age of the infant, the nature of the insult, and the intervention types. Studies of the effect of hypoxemia on brain energy metabolism in the immature rat brain have delineated a particular window of vulnerability, characterized by greater vulnerability in the second postnatal week, comparable to the human brain at term than in the first postnatal week, comparable to the human premature brain. In addition, based on the evidence of more resistance of cerebral energy metabolisms and longer duration of survival in the immature than in the adult brains submitted to asphyxia insults, there is a long-held general notion that the perinatal brain is more tolerant to asphyxia than the adult brain [4, 5]. However, neuropathological studies indicate that many critical neuronal groups are more vulnerable to HI injury in the immature animals, particularly related to enhanced density and function of excitatory amino acid receptors and enhanced vulnerability to attack by reactive oxygen species (ROS) and reactive nitrogen species (RNS) [5].

2.1. Major Neuropathology. The development of brain injury after HI insult is an evolving process imitated during the acute insult and extending into a reperfusion phase. The principle pathogenetic mechanism underlying neurological damage in HIE resulting from hypoxemia/ischemia or both is deprivation of glucose and oxygen supply which causes a primary energy failure and initiates a cascade of biochemical events leading to cell dysfunction and ultimately to cell death [6, 7]. A consequent reperfusion injury often deteriorates the brain metabolism by increasing the oxidative stress damage. Particular roles for increase in extracellular glutamate, excessive activation of glutamate receptors (excitotoxicity), increase in cytosolic calcium (Ca^{2+}), and generation of free radicals are emphasized.

The temporal aspects of the changes in glucose and energy metabolism after HI insult have been identified and include primary energy failure and secondary energy failure [8–13]. Immediately after HI insults, primary energy failure, depletion of oxygen precludes oxidative phosphorylation (decrease in high-energy phosphorylated compounds such as ATP and phosphocreatine) and results in a switch to anaerobic metabolism, causing accumulation of lactate and the associated H^+. The accumulation of lactate and the associated H^+ initially is beneficial for adoption to oxygen deprivation, but later, with progression of lactate formation, it has deleterious effects on (1) impairment of vascular autoregulation, a potential for advanced ischemic injury, (2) inhibition of phosphofructokinase activity by low pH, and (3) a biochemical cascade leading to cellular injury [8]. The occurrence of secondary energy failure varies according to species and nature of the insult with onset at appropriately 8∼16 hours and a nadir at 24∼48 hours. High-energy phosphate levels recovered to baseline levels in 2∼3 hours after reperfusion and reoxygenation, and a second decline in high-energy phosphate was pronounced at the next 48 hours [9, 11, 12].

An initial decrease in high-energy phosphate triggers a series of additional mechanisms, beginning with a failure of the ATP-dependent Na^+-K^+ pump. Transcellular ion pump failure results in the intracellular accumulation of Na^+, Ca^{2+}, and water (cytotoxic edema) followed by membrane depolarization, excessive release of excitatory neurotransmitters, specifically glutamate, increase of cytosolic Ca^{2+}, activation of phospholipase, and generation of free radicals. During the past 2 decades, remarkable studies have demonstrated the critical role for glutamate as the mediators of neuronal death in the HI insult [14–16]. Glutamate is the predominant excitatory amino acid neurotransmitter in the brain and has 3 major types of ionotropic receptors, NMDA, AMPA, and KA, as well as a group of G-protein-linked metabotropic glutamate receptor, existing in most neurons and glia possess. Normally, glutamate ionotropic receptors work cooperatively in stabilization of synapses and display a sequential participation in activity-dependent neuronal plasticity and neuronal excitation for normal tasks such as learning and memory. However, their vital role and enhanced function in the perinatal period also make neurons more vulnerable to excitotoxicity. Extracellular glutamate concentrations increase manifold with HI insults [17–19], and specific glutamate receptor channel blockers ameliorate brain injury in HIE [20]. The ontogeny of glutamate, transient dense expression of NMDA receptors and GluR2-deficient AMPA receptors, is relevant to the vulnerability of immature brain regions to excitotoxic cell death in HIE [21, 22]. The presence of a GluR2 subunit renders the AMPA receptors impermeable to calcium. The approximate time of peak sensitivity of excitotoxicity in rats is 6 days for NMDA and 9∼10 days for AMPA, appropriate to human prematurity and term newborn, respectively [23, 24]. Moreover, the topography of glutamate synapses, early expression of glutamate receptors in human hippocampus, cerebral cortex, and deep nuclear structures, is similar to regions vulnerable to HI injury in the newborn [18, 19, 25]. The increase in extracellular glutamate concentration and activation of glutamate receptors after hypoxia-ischemia triggers excitotoxic cascade. There is an increase in cytosolic Ca^{2+} by influx through open NMDA and calcium permeable AMPA receptor channels and other voltage-dependent Ca^{2+} channels, and the release of calcium from intracellular stores. The deleterious effects of increased cytosolic calcium include the activation of neuronal nitric oxide synthase (nNOS) to form nitric oxide, generation of free radicals, and degradation of cellular lipids by activation of phospholipases, of cellular proteins by activation of protease, and of cellular DNA by activation of nucleases, as well as accentuation of mitochondrial injuries [26–28]. Mitochondrial outer membrane permeabilization, in turn, elicits mitochondrial release of cytochrome C, activation cleavage of caspases 9 and 3, and apoptosis-inducing factor (AIF), leading to apoptosis [29]. The combined effects of cellular energy failure, acidosis, glutamate release, intracellular Ca^{2+} accumulation, lipid peroxidation, and nitric oxide neurotoxicity serve to disrupt essential components of the cell with its ultimate death. The mechanism of neuronal cell death after hypoxia-ischemia includes neuronal necrosis and apoptosis, depending principally on the severity of the insult and the maturational state of the cell. There is a continuum of necrosis and apoptosis, and often the early cell death appears necrotic and later cell death appears apoptotic [30, 31]. Initial decrease in high-energy phosphates will result in impairment of ATP-dependent Na^+-K^+ pump, which in the severe insult causes an acute influx of Na^+, Cl^-, and water with consequent cell swelling, cell lysis, and thus early cell death by necrosis whereas in less severe insult causes membrane depolarization followed by a cascade of excitotoxicity and oxidative stress leading to a delayed cell death, principally apoptosis. Apoptosis is more prevalent as a mode of death in the perinatal brain, and both caspase-dependent and caspase-independent mechanisms of apoptotic cell death have been recognized (Figure 1).

Experimental studies indicate that the first observable change in the neuron is cytoplasmic vacuolation, caused by mitochondrial swelling, occurring within 5∼30 minutes after the onset of hypoxia, and the differentiating oligodendrocytes exhibit approximately the same sensitivity to glucose and oxygen deprivation as do neurons. In the immature and mature brain, the order of cellular elements vulnerable to hypoxia-ischemia is neuron > oligodendroglia > astrocyte > microglia [32]. Yue et al. demonstrated that

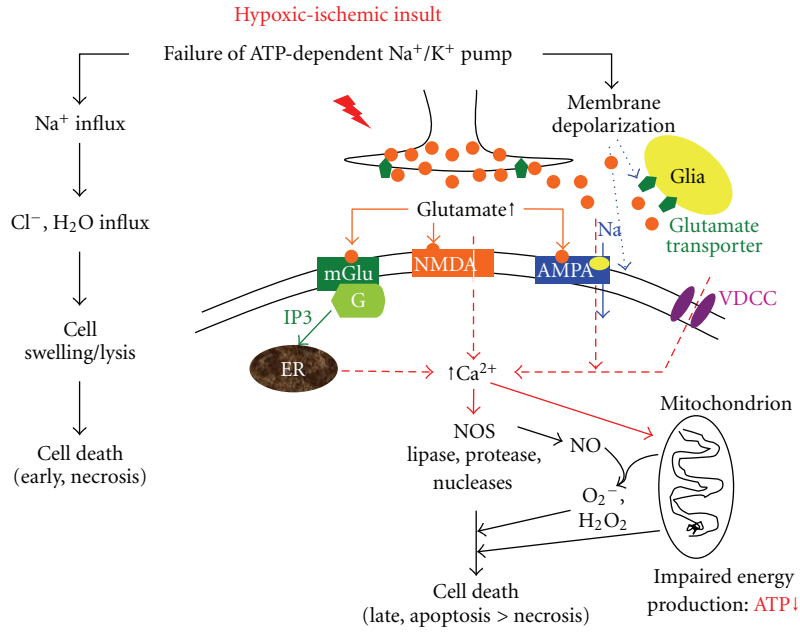

Figure 1: Proposed pathogenesis of hypoxic-ischemic encephalopathy. The central roles for ATP depletion, membrane depolarization, glutamate-mediated excitotoxicty, and voltage-dependent and glutamate-activated Ca^{2+} channels are apparent. An initial decrease in high-energy phosphates can result in an acute influx of Na^+, Cl^-, and water with consequent cell death (necrosis) in the severe insult, whereas in less severe insult, it causes membrane depolarization followed by a cascade of excitotoxicity and oxidative stress leading to a delayed cell death, principally apoptosis. Persistent membrane depolarization results excessive presynaptic glutamate release, reversal of glutamate transport in glia and neural terminals, and activation of NMDA and immature (GluR2 deficiency) AMPA receptors with profound Ca^{2+} influx with a series of Ca^{2+}-mediated cascades to cell death. The deleterious effects of cytosolic Ca^{2+} are multiple, including degradation of cellular lipids by activation of phospholipase and of cellular DNA by activation of nucleases and enhancement of generation of free radicals and nitric oxide (NO) by increase of nitric oxide synthase (NOS) [7, 8, 26]. AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; ER: endoplasmic reticulum; mGlu: metabotropic glutamate; NMDA: N-methyl-D-aspartic acid; NOS: nitric oxide synthase; VDCC: voltage-dependent calcium channels.

apoptotic neuronal death predominated among immature neurons, whereas necrotic cell death predominated among mature neurons [33]. The feature of neuropathology varies according to the developing age as hypoxia-ischemia insult. Three major regional patterns of selective neuronal necrosis in newborns with HIE, especially the term newborns, diffuse disease, cerebral-deep nuclear with prominent involvement of cerebral neocortex, hippocampus, and basal ganglia-thalamus, and deep nuclear-brain stem disease [34, 35]. The principal form of hypoxia-ischemic brain injury in the immature brain involves cerebral white matter, causing periventricular leukomalacia (PVL), and the data indicate an implication of a particular maturation-dependent intrinsic vulnerability of premyelinating oligodendrocytes (pre-OLs) to both endogenous and exogenous reactive oxygen species [35–37]. The immature brain has a propensity for ischemia to cerebral white matter because of the presence of (1) vascular end zones and border zones in that region and (2) impairment of cerebrovascular autoregulation [7]. Ness et al. noted a transition of cell death in white matter from early necrotic deaths to hybrid cell deaths to classical apoptosis between 4 and 24 h of recovery from hypoxia-ischemia [38]. The delayed time course of apoptosis in pre-OLs supports the feasibility of interventions to improve clinical outcomes for newborns surviving birth asphyxia.

3. Potential Therapies for Neonatal Hypoxic-Ischemic Encephalopathy

3.1. Supportive Intensive Care. Perinatal HIE is a major cause of death and disability worldwide which has been limited to supportive intensive care. It includes correction of hemodynamic and pulmonary disturbances (hypotension, metabolic acidosis, and maintenance of adequate ventilation), correction of metabolic disturbances of glucose, calcium, magnesium, and electrolytes, treatment of seizures if present, and monitoring for other organ system dysfunctions, such as acute renal failure. Maintenance of adequate ventilation and adequate perfusion is a central aspect of supportive care. Oxygen deprivation may lead to disturbance of cerebrovascular autoregulation with consequence of a pressure-passive cerebral circulation and increase additional neuronal and white matter injury. Severe hyperoxia in the first hours of life may contribute to increased oxidative stress, deleterious to long-term neurological consequences [39, 40], and the guideline of the 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with Treatment Recommendations (CoSTR) in the International Liaison Committee on Resuscitation (ILCOR) recommends that, for term babies, it is better to begin resuscitation with room air rather than

100% oxygen [41]. In addition, the maintenance of adequate perfusion to brain, more related to adequate arterial blood pressure than intracranial pressure, is important for prevention of additional ischemic injury.

3.2. Control of Seizures. The presence of seizure, practically occurring within the first hours, predicates a poor outcome of HIE. Therefore, antiepileptic drugs (AEDs) are among the medications most commonly used in perinatal HIE. The energy metabolism can be compromised by the hyperactive neurons, and both acute energy deprivation after HI insult and seizures are implicated in excitotoxicity. Thus, the therapeutic value of AEDs may include not only control of seizure activity but also potentially the benefit for the compromised cellular energy metabolism. Studies about perinatal HIE showed a beneficial effect of pretreatment with barbiturates [42, 43]. Phenobarbital remains the preferred drug for the treatment of seizures in neonates with HIE [7]. It is still a controversial issue whether phenobarbital treatment should be administered before the seizure attacks. Close observation with use of continuous EEG to identify seizures is optimal for management of the asphyxiated infants.

3.3. Neuroprotective Strategies. At present, no individual neuroprotective agents have been proven safe and effective for the protection of neonates from neurological sequels after HI insults. The insight into the biochemical and cellular mechanisms of neuronal injury with perinatal HIE helps to provide interventions to interrupt those deleterious cascades, principally focusing on the potential effects of free radical scavengers, such as N-acetylcysteine (NAC) and allopurinol, magnesium, glutamate receptor blockers, erythropoietin (Epo), and hypothermia. NAC is a free radical scavenger and has been demonstrated to minimize hypoxia-ischemia-induced brain injury in various acute models [44–46]. Combination therapy of NAC and systemic hypothermia improves infarct volume, myelin expression after focal HI injury [44]. Treatment of allopurinol, a xanthine oxidase inhibitor and free radical scavenger, also exerts benefit on reduction of cerebral edema and neuropathological damage after neonatal HIE [47]. Epo, the major haemopoietic growth factor, is now considered to have beneficial effects in various nervous system disorders based on the effects of prevention of metabolic compromise, neuronal and vascular degeneration, and inflammatory cell activation [48–50]. Gonzalez et al. demonstrated that the treatment of Epo preserves hemispheric brain volume after an occlusive cerebral injury in P10 rats [48]. Other experimental studies concerning the potential value of magnesium sulfate reveal that blockers of glutamate receptors have conflicting results. However, the most potent and promising intervention to prevent energy depletion is hypothermia. Experimentally, reducing body temperature to 3∼5°C below the normal level reduces cerebral injuries, including a decrease in brain energy utilization, reduction of infarct size, and amelioration of neuronal cell loss and hippocampal structure, and improves neurological outcomes after asphyxia [51–54]. The beneficial effects of mild hypothermia occur at multiple sites in the cascade to cell death. Hypothermia must be commenced before the onset of delayed energy failure and particularly excitatory features, such as seizures [7]. Early induction of hypothermia in human infants who had perinatal HIE improves survival and reduces the rate of disability of those survivors [41, 55–58]. The guideline of ILCOR CoSTR 2010 recommends therapeutic hypothermia as a standard practice for term to near term infants with moderate to severe HIE [41].

4. Conclusion

Hypoxia-ischemia in the perinatal period is a major cause of neonatal death and long-term disability. There are advances in research of cellular processes and molecular mechanisms underlying HIE over the last 2 decades. Hypothermia is the only treatment effective in neonatal HIE at present. Combined therapy of hypothermia and other neuroprotective strategies, focusing on prevention of acute injuries, increase of therapeutic time window, and enhancement of neural repair, is expected to improve the neurological outcomes of HIE.

Conflict of Interests

The authors declare that there are no conflict of interests.

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