Neonatal Hypoxic–Ischemic Encephalopathy: Perspectives of Neuroprotective and Neuroregenerative Treatments

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Abstract

Hypoxic–ischemic encephalopathy (HIE) is a serious condition that could have deleterious neurological outcomes, such as cerebral palsy, neuromotor disability, developmental disability, epilepsy, and sensitive or cognitive problems, and increase the risk of death in severe cases. Once HIE occurs, molecular cascades are triggered favoring the oxidative stress, excitotoxicity, and inflammation damage that promote cell death via apoptosis or necrosis. Currently, the therapeutic hypothermia is the standard of care in HIE; however, it has a small window of action and only can be used in children of more than 36 gestational weeks; for this reason, it is very important to develop new therapies to prevent the progression of the hypoxic–ischemic injury or to develop neuroregenerative therapies in severe HIE cases. The objective of this revision is to describe the emerging treatments for HIE, either preventing cell death for oxidative stress, excitotoxicity, or exacerbated inflammation, as well as describing a new therapeutic approach for neuroregeneration, such as mesenchymal stem cells, brain-derived neurotrophic factor, and gonadotropin realizing hormone agonists.

Keywords
- excitotoxicity
- hypoxic–ischemic encephalopathy
- inflammation
- neuroprotection
- neuroregeneration
- oxidative stress

Introduction

Hypoxic–ischemic encephalopathy (HIE) appears as a result of decreased oxygen and blood flow supply to the brain, the HIE incidence is estimated between 1.5 and 3 per 1,000 live births[1]; in moderate HIE, patients have a 10% risk of death and 60% of children with severe HIE die; while 30% of moderate HIE and almost all surviving children with severe HIE could have neurological outcomes such as cerebral palsy, neuromotor disability, developmental disability, epilepsy, and sensitive or cognitive problems.[2]

The standard of care for HIE is the therapeutic hypothermia (TH) which has demonstrated significant reductions in neurological outcomes when compared with controls.[3] However, hypothermia only can be used in term children or at least in children with 36 weeks' gestational age, it should be administered in the first 6 hours after the insult and requires special equipment that in low- or middle-income countries could be difficult to achieve due to the lack of infrastructure.[4]

There is a serious need for developing new treatments to prevent or correct the brain damage after a neonatal hypoxic–ischemic event. The objective of this revision is to describe the emerging treatments for HIE, either preventing cell death for oxidative stress, excitotoxicity, or exacerbated inflammation, as well as describing new therapeutic approaches for neuroregeneration.

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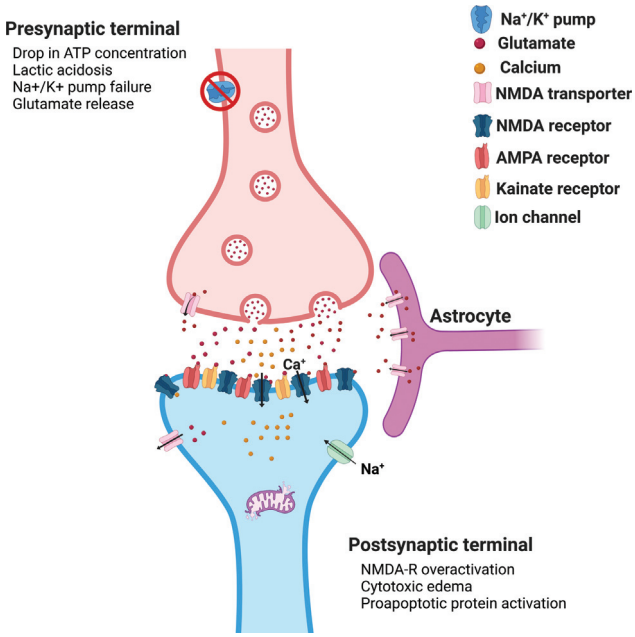

Fig. 1 Diagram of the excitotoxicity cascade involved in neonatal hypoxia–ischemic encephalopathy. Ca^{2+}, calcium ion; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA, N-methyl-D-aspartate.

Molecular Cascades of Hypoxic–Ischemic Encephalopathy

The deleterious effects of HIE are triggered by three mainly damaged cascades, excitotoxicity, oxidative stress, and exacerbated inflammation, those together can generate apoptosis or necrosis of neurons and glia cells in different phases of the injury.

Initially, the lack of blood and oxygen generates an acute energetic failure in the cells because of the drop in adenosine triphosphate (ATP) concentration that switches cells metabolism from aerobic to anaerobic producing lactic acidosis. The ATP deficit causes Na^+/K^+ pump failure, as a result, there are sodium, calcium, and water intracellular accumulation, followed by neuronal depolarization and glutamate release. The glutamate is an excitatory amino acid that over activates postsynaptic receptors, such as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainite receptors, allowing the entry of calcium and sodium to the postsynaptic cell and in severe cases cell necrosis or apoptosis because of the excitotoxicity[5,6] (►**Fig. 1**).

Inflammation is another main component of brain damage during hypoxia–ischemia. The microglia are activated and migrate to the area of damage, producing proinflammatory cytokines, glutamate, nitric oxide (NO), and free radicals. Similarly, astrocytes participate in the inflammatory response, favoring the permeability of the blood–brain barrier (BBB) and allowing the crossing of leukocytes such as neutrophils, macrophages, and T-lymphocytes[7,8] (►**Fig. 2**).

Finally, the period of reperfusion that continues a hypoxic–ischemic event is associated with an accelerated increase in free radical production and oxidative stress (►**Fig. 3**), neonatal brain is highly susceptible to oxidative damage because of the immature antioxidant defense, higher concentrations of free iron, and high amounts of fatty acids.[9] Superoxide (O_2^-) production is mediated for the increase in intracellular oxygen and escape of electrons from mitochondrial complexes I and III. O_2^- dismutase (SOD) converts O_2^- into hydrogen peroxide (H_2O_2) to reduce the oxidizing effect of the O_2^- radical, subsequently H_2O_2 is converted in water by catalase.[10] Neonatal brain has an immature antioxidant

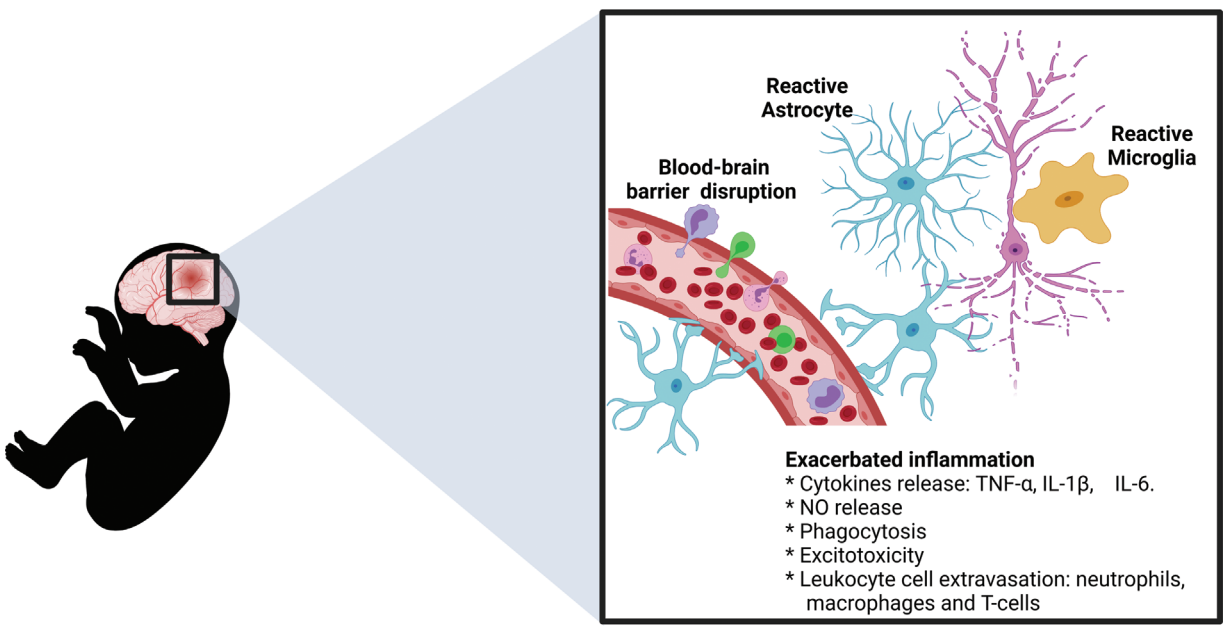

Fig. 2 Role of inflammation in hypoxic–ischemic encephalopathy. IL, interleukin; NO, nitric oxide; TNF-α: tumor necrosis factor-α.

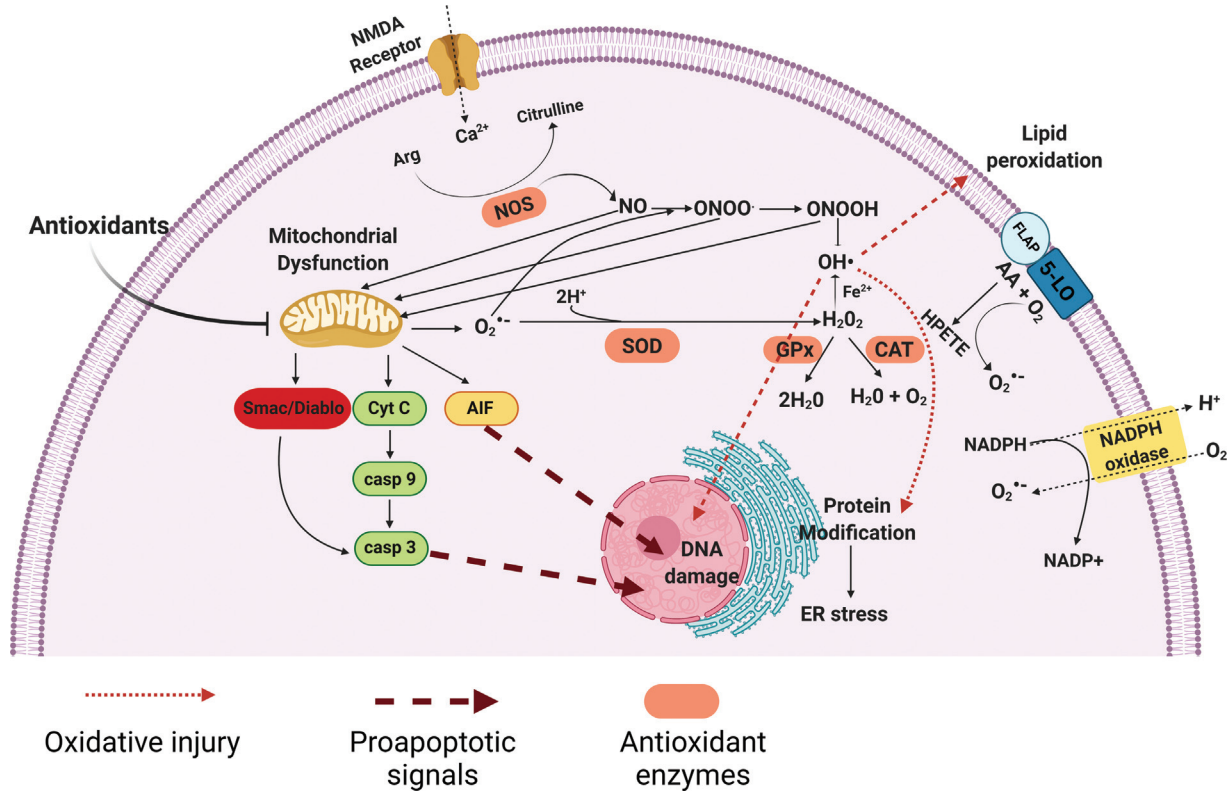

Fig. 3 Representative diagram of oxidative stress cellular injury in hypoxic ischemic encephalopathy. ARA, arachidonic acid; AIF, apoptosis-inducing factor; Arg, arginine; casp 3, caspase 3; casp 9, caspase 9; Cyt C, cytochrome C; DNA, deoxyribonucleic acid; ER: endoplasmic reticulum; Fe^{2+}, iron; FLAP, 5-lipooxygenase-activating protein; Gpx, glutation peroxidase; H_2O_2: hydrogen peroxide; H^+, hydrogen; HPETE, hydro-peroxy-eicosatetraenoic acid; NADPH, nicotinamide-adenine dinucleotide phosphate; NO, nitric oxide; $ONOO^-$, peroxynitrite; ONOOH, NOS, nitric oxide synthase; O_2, molecular oxygen; $O_2\bullet^-$, superoxide; 5-LO, 5-lipooxygenase.

defense with less production of catalase, this situation increases H_2O_2 concentration that in presence of iron by Fenton's reaction produces the hydroxyl radical (OH^-) a potent free radical. Free radicals also stimulate membrane phospholipase activity during ischemia, causing the release of polyunsaturated fatty acids (PUFA). The oxidation of PUFA via the cyclooxygenase and lipoxygenase pathways forms thromboxanes, prostaglandins, and leukotrienes induces chemotaxis, inflammation, and more oxygen-free radicals.[11] The overactivation of NMDA receptor and induced calcium influx increasing the activation of neuronal NO synthase (nNOS) to form NO. NO in presence of O_2^- yield peroxynitrite ($ONOO^-$) which initiates lipid peroxidation, enhances glutamate release, and leading generation of OH^-[12] and increased permeability of the BBB. BBB dysfunctions allow the passage of large proteins into the extracellular space, increasing brain inflammation and degradation of regulatory proteins in the vascular basement membrane because of metalloproteases.[6,12] In ►**Fig. 4**, the stages of the hypoxic–ischemic brain injury are represented.

Neuroprotective Therapeutics for Hypoxic–Ischemic Encephalopathy

The increase in reactive oxygen and nitrogen species (ROS and RNS, respectively) after hypoxia–ischemia produces oxidative stress is an important damaging factor that results in brain necrosis and apoptosis. In addition, the excessive release of the neurotransmitter glutamate has a cytotoxic effect. The exacerbated response of immune cells favors damage after days or weeks of the hypoxic–ischemic insult, thus emerging treatments are aimed at preventing the three main mechanisms of damage.[13]

Allopurinol

Allopurinol is a xanthine oxidase (XO) inhibitor that reduces the production of uric acid and scavenges free radicals.[14] In high concentrations, it acts as an iron chelator.[15] In addition, it has few adverse effects, such as skin rashes and hypersensitivity reactions, which do not represent a serious health risk.[16]

The efficacy of allopurinol on preventing brain damage was evaluated in animal models. Palmer et al evaluated the administration of allopurinol (135 mg/kg subcutaneously) 15 minutes after a hypoxic–ischemic event in rats at postnatal day 7, demonstrating reduced acute brain edema and long-term cerebral injury.[17] In other study on hypoxic–ischemic piglets, the administration of allopurinol (30 mg/kg/d intravenously) reduced the phosphocreatine/inorganic phosphate ratios, indicating preservation of energy status. The allopurinol treatment reduced vasogenic edema,[18] possibly due to smaller decrease in Na^+/K^+ ATPase activity.[19] However, it was not

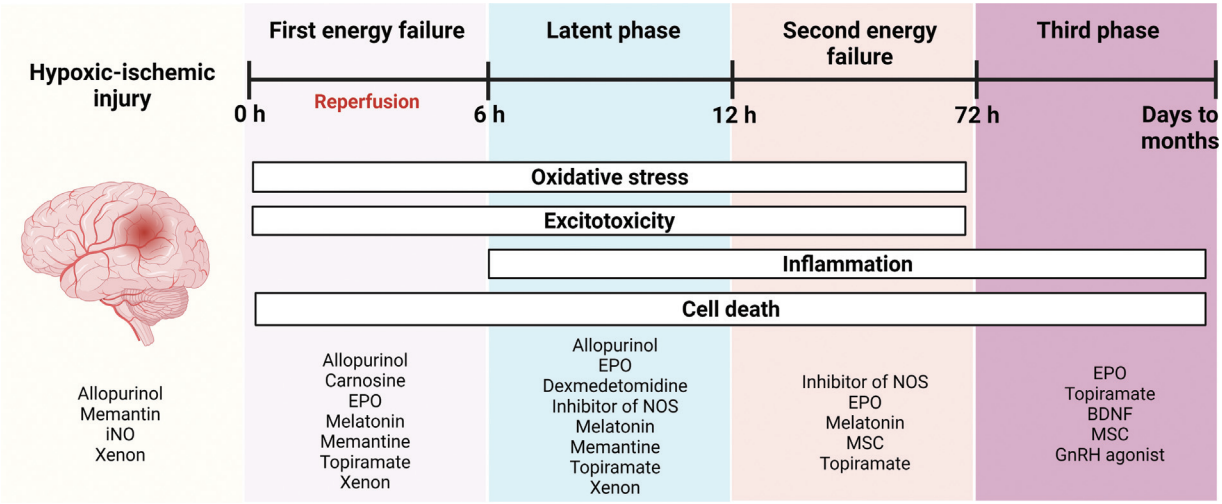

Fig. 4 Stages of hypoxic-ischemic brain injury and possible neuroprotective and neuroregenerative therapies. BDNF, brain-derived neurotrophic factor; EPO, erythropoietin; GnRH agonists, gonadotropin-releasing hormone agonists; iNO, inhaled nitric oxide; inhibitor NOS, inhibitor of nitric oxide synthase; MSC, mesenchymal stem cells.

effective in preventing cell death since similar results were observed in caspase-3 activity and terminal transferase dUTP nick end labeling (TUNEL) labeling when compared with placebo.[18] When the administration of allopurinol is made together with TH, it seems to work synergistically reducing the infarct area and decreasing caspase-3 activity.[20]

Preclinical studies served as the basis for evaluating the effectiveness of allopurinol in the treatment of HIE in newborns. Gunes et al demonstrated that newborns severely asphyxiated had increased serum and cerebrospinal fluid concentrations of NO but infants who received allopurinol, significantly decrease of serum NO concentration recorded after 72 to 96 hours of birth, whereas cerebrospinal fluid NO concentrations also decreased but not significantly. This shows the scavenging effect of allopurinol, and no adverse side effects of allopurinol use were reported in that study.[21]

Compared with controls, allopurinol long-term effects on intelligence and neurological outcomes were no different after 4 to 8 years of follow-up.[22] Those results are confirmed by Chaudhari and McGuire who suggested the need for larger trials, and registration of clinically important effects, mortality, and morbidity of allopurinol as a joint therapy of TH.[23] Consequently, in Europe, a double-blind, placebo-controlled, phase-III study is currently performed (ALBINO, identifier: NCT03162653), with the purpose of evaluating the efficacy and safety of early postnatal allopurinol treatment in HIE.[24]

Some results about the pharmacokinetic and pharmacodynamics of allopurinol in neonates with HIE were published.[25] The authors compared samples of neonates from the ALBINO study (HIE neonates with and without TH) and neonates from two historical studies performed by van Bel et al[26] and Benders et al.[27] Neonates from ALBINO study received allopurinol 20 mg/kg within 45 minutes after birth ($n = 20$), and neonates under TH ($n = 13$) received a second dose of allopurinol 10 mg/kg, 12 hours later. Neonates from the two historical studies did not receive TH, instead received two doses of allopurinol 20 mg/kg, first dose was administered within 4 hours after birth and the second dose at 12 hours later ($n = 26$). When compared, the clearance of allopurinol and its active metabolite oxypurinol were similar between TH and non-TH patients. Besides, allopurinol and oxypurinol inhibited XO > 90% during the first 24 hours, with a concentration at the half maximal XO inhibition of 0.36 mg/L (95% confidence interval [CI]: 0.31–0.42); demonstrating that the regimen treatment for allopurinol not required adjustment in the ALBINO study.[25]

The frame window for allopurinol administration could be a determinant for neuroprotective results, as demonstrated by Benders et al who did not find changes in mortality or adverse neurological outcomes in severely hypoxic neonates after allopurinol treatment (40 mg/kg intravenously divided in two doses), the authors proposed that the administration of allopurinol within 4 hours after birth was too late since hypoxic induction of XO and the subsequent oxidative stress occur immediately after reoxygenation.[27] Also reported therapeutic concentrations of allopurinol in the cord blood after the administration of 500 mg of allopurinol in mothers in labor with risk of fetal hypoxia.[27] Because of that, Boda suggested to implement controlled trials to determine the prophylactic effect of allopurinol in mothers with deliveries at risk of developing hypoxia.[28] If proven effective and safe, allopurinol could become a part of neuroprotective drug treatment strategy in addition to TH or as a prophylactic therapy.

Carnosine

Carnosine is an endogenous dipeptide (β-alanyl-l-histidine) abundant in excitable tissues, including skeletal and cardiac muscle and nervous tissue. It has properties as a pH buffering, neutralizing the improved formation of lactic acid in anaerobic exercise,[29,30] and also exerts antioxidant activities as a ROS and RNS scavenger, chelator of zinc and copper ions, and protein glycosylation inhibitor.[31]

Carnosine can easily cross the BBB and has been demonstrated such a safety agent in rat models of HIE. Carnosine

antioxidant actions have been proposed as a neuroprotective agent in the treatment of HIE before[32] and after the injury.[33]

To explain the carnosine mechanism for neuroprotection, neurons and astrocytes cultures were exposed to oxygen/glucose deprivation. Carnosine reduced neuronal cell death, correcting excitotoxicity through the upregulation of glutamate transporter and gamma-aminobutyric acid levels, and reversing mitochondrial energy metabolism damage.[34] In a mouse model of permanent focal cerebral ischemia, carnosine downregulated matrix metalloproteinases activity and reduced ROS and RNS, decreasing infarct size and neuronal damage.[35] It is striking that carnosine in combination with TH effectively reduced the extent of brain damage; however, this result was not observed with the only administration of carnosine.[36]

The neuroprotective effect of carnosine may be dose dependent. In a study of chronic cerebral ischemia in mice, it was observed that a dose range of 200 to 500 mg/kg of carnosine protects against white matter damage likely by reducing oligodendroglia cell loss, but this result was not observed in a higher dose of 750 mg/kg.[37] Future clinical trials are needed to evaluate the safety and effectiveness of carnosine for HIE treatment.

Dexmedetomidine

Dexmedetomidine is a high selective α_2 adrenoreceptor agonist. Its clinical applications are sedation, analgesia, anxiolytic, and opioid-sparing effects with the benefit of less respiratory depression than other sedatives. In high concentrations, it has been reported side effects like bradycardia, hypotension, and hypertension.[38] Despite the adverse effects, dexmedetomidine is considered a neuroprotective drug. In a rodent model of HIE, it was demonstrated its participation in neuroglobin upregulation. Neuroglobin is a hemoprotein with high affinity to oxygen that also has ROS and RNS scavenging actions. The effect of dexmedetomidine on neuroglobin had a surviving effect on nerve cells after a hypoxic–ischemic event, probably achieved by downregulation of cytosolic cytochrome-c, apoptosis protease-activating factor-1 (Apaf-1), and caspase-3, thus inhibiting neuronal apoptosis through the mitochondrial pathway.[39]

The immunomodulatory role of dexmedetomidine has been described by decreasing the expression of tumor necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) in a rat model of HIE.[40] Antiexcitotoxic actions of dexmedetomidine were described in a murine model of perinatal excitotoxic injury decreasing the size of lesion in cortex and white matter.[41]

In addition, dexmedetomidine pharmacokinetics and safety were evaluated in a phase-I study, where seven neonates with moderate or severe HIE were included. Dexmedetomidine was infused during TH and the 6 hours rewarming period, starting at 0.2 µg/kg/h and reached the steady state of 0.4 µg/kg/h after 2.5 hours. The dose employed was safe since no acute adverse effects were reported. Pharmacokinetics of dexmedetomidine showed delayed clearance 0.760 L/kg/h, and longer steady state distribution volume of 5.22 L/kg and longer elimination half-life of 7.3 hours compared with normothermic neonates without HIE with an elimination half-life of 3 hours.[42] Future clinical trials are needed to evaluate the effectiveness of dexmedetomidine as a neuroprotective for HIE.

Erythropoietin

Erythropoietin (EPO) is a glycoprotein produced by the kidney that stimulates the bone marrow for erythropoiesis but also has another extrahematopoietic functions.[43] In brain hypoxia, the hypoxia inducible factors 1 and 2 (HIF 1 and 2) increases EPO synthesis and the expression of its receptor (EPO-R) to improve red-cell mass and tissue oxygenation.[43] It has been demonstrated in animal studies that EPO-R increases its expression at brain capillaries after hypoxia ischemia, suggesting an improvement in the passage of this hormone through the BBB.[44]

The neuroprotective effect of the EPO includes antiapoptotic effect, presumably for the downregulation of Bax and DP5 proapoptotic gene expression[45]; decreased damage induced by NO,[46,47] antioxidant enzymes activation,[48] excitotoxicity reduction by inhibiting overactivation of NMDA receptor,[47,49] anti-inflammation,[50] neurovascular remodeling, and neural stem-cell proliferation.[51]

The effectiveness of EPO in the treatment of HIE has been proven in clinical trials, either as a single treatment or together with TH showing encouraging results (►**Table 1**). The EPO doses used for the treatment of HIE in clinical trials range from 250 to 2,500 U/kg, in single administrations or for up to 6 days.[46,52–57]

Monotherapy of recombinant human EPO (RH-EPO) at low doses (300 or 500 U/kg) for 5 to 6 days, diminished the risk of disability and cerebral palsy in newborns with moderate-to-severe HIE, and no negative hematopoietic side effects were reported.[52,57] Meanwhile, the dose of 1,000 U/kg administered for 5 days has been demonstrated to increase plasma EPO concentrations like those reported as ideal for the treatment of HIE in animal models.[53,54,56] The dose of 1,000 U/kg also is recommended in patients with TH and EPO cotreatment due to increased time for drug clearance after hypothermia.[56]

Monotherapy with RH-EPO at higher doses, like 2,500 U/kg for 5 days, has shown diminished NO concentration, improved electroencephalography (EEG) background and less risk of rehospitalization.[46] However, these results should be taken with caution, as this dose was tested in cases of mild-to-moderate HIE and not in severe cases, more studies are needed to see its safety in severe HIE.

It seems that for effective neuroprotection with EPO treatment, it is needed the administration of several doses, since a single dose of 1,500 U/kg compared with TH treatment did not show any improvement.[55] More studies are still needed to establish a standardized dose and duration of the treatment for better results in patients with moderate-to-severe HIE. However, it should be ensured that the selected dose does not cause serios adverse effects as venous thromboembolism,[58] polycythemia (hematocrit >60 or hematocrit increase $\geq 15\%$), hypertension, intraparenchymal or grade III/IV intraventricular hemorrhage, or unexpected death[56]; until now, the doses proved that

Table 1 Clinical trials for HIE treatment with erythropoietin

Country	Sample size	Treatment	Outcomes	
			Primary outcomes	Secondary outcomes
India[52]	100 neonates with moderate or severe HIE divided in two groups: 1. HIE-EPO group ($n = 50$) 2. HIE-placebo group ($n = 50$)	HIE-EPO received: RH-EPO within 6 hours after birth at a dose of 500 U/kg intravenously on alternate days for a total of five doses HIE-placebo group: 2 mL of normal saline on the same schedule	HIE-EPO group: eight patients died and 12 survived with severe or moderate disability HIE-placebo group: eight patients died and 27 survived with severe or moderate disability	Outcomes at 19 months: Less risk of cerebral palsy, less use of anticonvulsant treatment and less abnormalities in neonatal brain MRI in EPO group
United States of America[54]	50 neonates with moderate or severe HIE underwent hypothermia therapy in two groups: 1. HIE-EPO group ($n = 24$) 2. HIE-placebo group ($n = 26$)	HIE-EPO group: received RH-EPO 1,000 U/kg intravenously, at 1, 2, 3, 5, and 7 days of age HIE-placebo group: received an equal volume of normal saline the same days	HIE-EPO group: three patients died HIE-placebo group: six patients died Death was more common after severe encephalopathy	Brain MRI at mean 5.1 days showed a lower global brain injury score in EPO group Outcomes at 12 months: higher AIMS and WIDEA score in EPO group
Egypt[55]	45 neonates in four groups: 1. Healthy group ($n = 15$) 2. HIE-EPO group ($n = 10$) 3. HIE-hypothermia ($n = 10$) 4. HIE-supportive care ($n = 10$)	HIE-EPO group: single subcutaneous 1,500 U/kg RH-EPO at day 1 HIE-hypothermia for 72 hours	HIE-EPO group: seven patients died HIE-Hypothermia: four patients died HIE-supportive care: eight patients died	Therapeutic hypothermia was superior to single dose RH-EPO for neuroprotection in HIE especially in patients with stage-II Sarnat's scale
United States of America[53,56]	24 neonates with moderate or severe HIE that underwent hypothermia therapy divided into four groups: 1. HIE-EPO 250 ($n = 3$) 2. HIE-EPO 500 ($n = 6$) 3. HIE-EPO 1,000 ($n = 7$) 4. HIE-EPO 2,500 ($n = 8$)	Newborns received six doses of RH-EPO, first dose was administered before 24 hours of age and subsequent doses were given at 48-hour intervals Each patient received 1 of 4 dosages of EPO for all of their doses: • HIE-EPO 250 U/kg • HIE-EPO 500 U/kg • HIE-EPO 1,000 U/kg • HIE-EPO 2,500 U/kg	No serious adverse events nor neonatal deaths The dose 1,000 U/kg intravenously was well tolerated and produces plasma concentrations similar than in animal models with neuroprotection	Outcomes at mean age of 22 weeks[53]: One child had quadriplegic cerebral palsy One child had hemiplegic cerebral palsy One child had epilepsy Three children had language problems One child had increased tone
Egypt[46]	45 neonates in three groups: 1. Healthy group ($n = 15$) 2. Mild or moderate HIE-EPO group ($n = 15$) 3. Mild or moderate HIE-placebo group ($n = 15$	Healthy group: no treatment HIE-EPO group: received RH-EPO 2,500 U/kg, subcutaneously, daily for 5 days HIE-placebo group: received an equal volume of normal saline	Rates of survival did not differ between groups NO concentrations at baseline were significantly increased in both the HIE groups compared with the healthy group	Outcomes at 2 weeks: decreased serum NO concentration and improved EEG backgrounds in EPO group Outcomes at 6 months: less altered DDST II results, less abnormal neurologic examination and rehospitalization

(*Continued*)

Table 1 (*Continued*)

Country	Sample size	Treatment	Outcomes	
			Primary outcomes	Secondary outcomes
China[57]	158 neonates with moderate or severe HIE divided into three groups: 1. HIE-control group ($n = 82$) 2. HIE-300 EPO group ($n = 47$) 3. HIE-500 EPO group ($n = 29$)	HIE-control group: conventional treatment HIE-300 EPO group: RH-EPO 300 U/kg subcutaneously the first time and then intravenously every other day for 2 weeks, starting <48 hours after birth HIE-500 EPO group: RH-EPO 500 U/kg on the same schedule	HIE-control group: four patients died HIE-EPO groups: three patients died	Outcomes at 18 months: less disability, cerebral palsy, and less proportion of scores MDI < 70 in EPO group

Abbreviations: AIMS, Alberta Infant Motor Scale; DDSTII, Denver Developmental Screening Test II, EEG, electroencephalography; EPO, erythropoietin; HIE, hypoxic–ischemic encephalopathy; MRI, magnetic resonance imaging; NO, nitric oxide; RH-EPO, recombinant human erythropoietin; WIDEA, Warner Initial Developmental Evaluation; MDI, mental developmental index.

they have not caused serious adverse effects in newborns with HIE treated with EPO.[59]

EPO treatment has the advantage of less technical complexity, less side effects, and has a greater window of action compared with TH which should be started within the 6 hours after the insult. EPO administration can be performed up to 48 hours after the birth.[57]

On the other hand, some clinical trials have not reported conclusive results. Wu et al mentioned that, due to the exclusion of two patients, statistical significance was lost between the results of placebo group and EPO.[54] Furthermore, the protective actions of EPO in overall disability were significantly reduced after EPO treatment for girls but not for boys which could indicate a dimorphism in the effectiveness of this treatment. In addition, the effectiveness of EPO treatment in improved long-term outcomes is only useful in cases of moderate HIE but not in severe HIE.[57]

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleaminic hormone produced by the pineal gland, working as a circadian pacemaker and with another pleiotropic bioactivities[60] such as a highly effective antioxidant, free radical scavenger,[61] anti-inflammatory, and antiapoptotic[62] and antiexcitotoxic effects.[63] In addition, due to its lipophilic properties, melatonin can easily diffuse through the BBB, giving it neuroprotective actions.[64]

Melatonin is synthesized from the amino acid precursor tryptophan, primarily by the pineal gland, but also can be produced for cells with aerobic activity and the intracellular concentrations that are higher than those circulating in blood.[64,65] Its half-life in serum is between 30 and 60 minutes,[60] in humans, it has two types of receptors, MT1 and MT2 members of the G-protein coupled receptors family, both receptors are in different tissues including brain, brown, and white adipose tissue, coronary artery, pancreas, granulose cells, myometrium, fetal kidney, testis, and the retina.[66]

The neuroprotective role of melatonin in HIE has been described in animal models by the prevention of damage at different stages of the injury after a hypoxic–ischemic event.[64] Melatonin exhibits an antioxidant role, also participates in mitochondrial damage prevention, and has anti-excitotoxic, antiapoptotic, and anti-inflammatory effects.[67] In addition, melatonin favors the survival of neurons and other glial cells, such as astrocytes, that is, important cells in the conservation of the BBB integrity.[68]

The antioxidant activity of melatonin in HIE consist in reducing the synthesis and increasing the scavenging of ROS and RNS.[60,69] Melatonin also exerts its antioxidant effect increasing the activity and expression of antioxidant enzymes such as O_2^-SOD, catalase, glutathione peroxidase, and glutathione reductase.[70] Balduini et al demonstrated, in a rat model of HIE, that melatonin administration after a hypoxic–ischemic event decreases oxidative protein damage[71] by downregulation of inducible NOS.[72]

Melatonin also could promote cell survival by keeping down cytochrome-*c* and apoptosis-inducing factor (AIF)

release to cytoplasm, preventing mitochondrial pore formation and the activation of intrinsic apoptotic pathways after the hypoxia–ischemia.[73] Melatonin also could prevent autophagy, in a study of rats with HIE who receive melatonin before the insult, and a markedly attenuated LC3-positive neuron's expression was observed. LC3 was a specific marker for autophagy; furthermore, melatonin effectively reversed the reduction of P62, a specific substrate that is degraded through the autophagy-lysosomal pathway.[74] In addition, it has been proven that melatonin prevents excitotoxic death of astrocytes.[61] Finally, melatonin exerts anti-inflammatory activity inhibiting cyclooxygenases, reducing inflammatory cells' recruitment and glial cells' activation in cerebral cortex.[71] The suppression of mRNA and protein expression of TNF-α and the intercellular adhesion molecule-1 (ICAM-1) could be the pathways of melatonin on lower infiltration of inflammatory cells into the damaged neural tissue.[61]

The above findings served as groundwork for melatonin's clinical trials use. First studies evaluated the pharmacokinetics of melatonin administration in preterm neonates. Merchant et al found that intravenous infusion of 0.1 µg/kg for 2 hours, reached melatonin plasma concentration of 203.3 pg/mL similar to that found in adult concentrations but clearance (0.045 L/h) and half-life time (16.91 hours) were prolonged.[75] Meanwhile, Carloni et al evaluated intragastric melatonin administration in three different schemes (0.5 mg/kg of melatonin, or three intragastric doses of 1 or 5 mg/kg in 24-hour intervals) and reported maximal serum concentrations of 0.44, 1.03, and 7.04 µg/mL, respectively. In addition, the half-life time (10.94, 9.37, and 7.98 hours, respectively) and clearance (31.19, 94.93, and 61.03, respectively) also were prolonged compared with adults.[76] However, both studies showed that melatonin concentration was reached to evaluate its effectiveness as a treatment for HIE.

In a study with 30 term newborns with moderate or severe HIE, the efficacy of melatonin combined with TH ($n = 15$) versus TH alone ($n = 15$) was evaluated; it was shown that five daily enteral doses of melatonin 10 mg/kg act synergistically with hypothermia, decreasing NO serum concentration, less seizures on follow-up electroencephalography, and less white matter abnormalities on magnetic resonance imaging. In addition, there were no side effects, and at 6 months, the melatonin/hypothermia group had improved survival without neurological or developmental abnormalities.[77] Similar survival results were found by Ahmad et al in preterm and term neonates (≥34 weeks of gestation) with mild or severe HIE. Also, 80 newborns were admitted at the study, 40 received standard treatment (oxygen therapy, intravenous fluids, intensive monitoring, and broad-spectrum antibiotic), and the rest 40 received standard treatment plus melatonin in a single dose of 10 mg via nasogastric. Melatonin therapy demonstrated greater survival rate in moderate and severe neonates with HIE.[78]

Despite these results, controversy still exists regarding the dose and the length of melatonin treatment for optimal neuroprotection. Balduini et al tested the pharmacokinetic, safety, and effect of melatonin administration in 5 newborns with moderate or severe HIE under TH. Melatonin was infused at a dose of 0.5 mg/kg via orogastric tube, starting 1 hour after reaching standard hypothermia temperature (33.5°C). Pharmacokinetics of melatonin showed serum concentration of 0.25 µg/mL, and prolonged elimination half-life of 26.4 hours and clearance of 0.21 L/h compared with adults, authors recommended intravenous administration because of the total bioavailability compared with enteral administration.[79]

Memantine

Memantine (1-amino-3,5-dimethyladamantane hydrochloride) is an uncompetitive open-channel blocker of NMDA receptors that has a fast response kinetics, sparing normal synaptic activity but inhibiting overactivation of NMDA receptors during excitotoxicity. Since NMDA receptors participate in the induction of long-term potentiation, the adverse effects of memantine could be related with learning and memory deficits, hallucinations, and sedation when is administered.[80] However, the only side-effects reported in clinical trials by memantine use were dizziness and restlessness/agitation at higher doses (40 mg/day).[81]

Neuroprotective activity of memantine has been proved in the *in vitro* and *in vivo* studies.[82] Rat cortical and retinal ganglion neuronal cells cultures treated with 6 to 12 µM of memantine expressed antiexcitotoxic activity by NMDA receptor blockade. On the other hand, administration of 20 mg/kg of memantine 1 hour prior to hypoxic–ischemic injury and 1 mg/kg maintenance dose every 12 hours diminished brain infarct size in a neonatal rat model of hypoxic–ischemic injury.[83] Similar results were found in an adult stroke model with approximately 30% less infarct size with a memantine dose of 20 mg/kg administered 2 hours after the injury. While memantine administration 1 hour after the hypoxic–ischemic event in rat pups with HIE diminished 28% the brain infarct size.[80] Memantine also demonstrated to protect developing and mature oligodendrocytes after a hypoxic–ischemic event in a rat periventricular leukomalacia (PVL) model.[84]

Memantine (20 mg/kg loading dose and 1 mg/kg every 12 hours for 2 days) has been proved as a combined therapy with topiramate (40 mg/kg loading dose and 10 mg/kg every 12 hours for 2 days) in a rat model of hypoxic–ischemic brain injury, demonstrating preservation of the brain mass and neurofunctional activity.[85] In addition, Landucci et al also evaluated combined therapies of memantine, topiramate, and hypothermia in the *in vitro* and *in vivo* models of HIE, describing enhanced protective effect when administered together, but memantine was especially effective to diminish brain infarct size in the rat HIE model when administered alone (20 mg/kg intraperitoneal).[86]

The use of memantine in preclinical studies of HIE seems promising; however, clinical studies are still needed to evaluate its safety and efficacy in the treatment of neonates with HIE. Currently memantine is used in the treatment of Alzheimer's, dementia, and other conditions with excitotoxic damage due to overactivation of the NMDA receptor.[81] The development of randomized double-blind clinical trials will

be important to evaluate its therapeutic application in HIE newborns.

Nitric Oxide–Targeted Therapy

NO is a gas that participates as a neurotransmitter, a signaling molecule, a radical scavenger, a vasodilator, and bronchodilator agent, but also participates as a free radical.[87] The brain production of NO is mediated by three isoforms of NOS, the endothelial (eNOS), nNOS, and inducible (iNOS), in which precursor is the amino acid L-arginine. The iNOS is activated under inflammatory conditions, while eNOS and nNOS are activated by Ca^{2+} and calmodulin binding.[88] As mentioned before, NO production is enhanced during reperfusion period after asphyxia, due to the overactivation of NMDA receptor and increased Ca^{2+} influx to the cell and is related with oxidative damage.[12]

Due to bronchodilator and vasodilator activity of NO, inhaled NO (iNO) has been used in the treatment of pulmonary hypertension by increased production of cyclic guanosine monophosphate (cGMP) inducing relaxation of smooth muscle.[89]

Preclinical studies have evaluated the efficacy of iNO in the treatment of hypoxic–ischemic brain injury.[90] Charriaut-Marlangue et al observed that infusion of 20 ppm of iNO during ischemia resulted in enhanced concentration of NO in the cortex, increased blood flow and reduced infarct volume (43%).[91] However, the dose and time of iNO administration is important for neuroprotection, doses $\geq$40 ppm during ischemia have shown exacerbated injury,[92] and similar deleterious results were found if iNO is administered after reperfusion period.[91,92] In addition, it seems that iNO therapy has better results in males, since administrations of 50 ppm during hypoxia had diminished brain injury in a mouse model of HIE but the neuroprotective effect was not present in females.[93]

Fukuda et al evaluated the feasibility and long-term outcomes of iNO therapy combined with TH in neonates with moderate-to-severe HIE and persistent pulmonary hypertension. In total, 37 newborns underwent TH of which 6 received iNO, the iNO infusion was safe, although two newborns discontinued TH because of intraventricular hemorrhage and uncontrollable pulmonary hypertension. At the 18 months of follow-up, there were no statistical differences among the Developmental Quotient and Gross Motor Function Classification system between children treated with TH or TH plus iNO.[94] Results of iNO therapy in newborns with HIE are insufficient and more clinical trials are needed to evaluate the safety and neuroprotection of iNO therapy in neonates receiving TH.

On the other hand, due to the neurotoxic properties of NO during reperfusion period, the use of selective inhibitors of NOS also has been probed. One advantage of the use of inhibitors of NOS is the longer time frame of up to 48 hours.[95] Favié et al evaluated the pharmacokinetics and safety of 2-iminobiotin, a selective inhibitor of nNOS and iNOS, in newborns with HIE treated with TH.[96] Authors expected to reach an area under concentration time curve from 0 to 48 hours (AUC_{0-48h}) of 4,800 ng/h/mL based on preclinical neuroprotective studies in piglets.[97] Results showed that administration of eight doses of 2-iminobiotin 0.08 mg/kg every 6 hours reached the targeted concentrations and were safety in cotreatment with TH, since no adverse effects with 2-iminobiotin were reported.[96] There are still doubts regarding the appropriate dose and treatment duration, as well as long-term outcomes after its use, more clinical randomized trials are needed to determinate its efficacy as a neuroprotective therapy.

Topiramate

Topiramate is an anticonvulsant drug that acts inhibiting several isoenzymes of carbonic anhydrase, also reduces excitatory neurotransmission by inhibiting voltage-gated sodium and calcium channels; the postsynaptic terminal reduces the excitability of AMPA and kainate glutamate receptors and modulates inhibitory neurotransmission via the gamma-aminobutyric acid ($GABA_A$) receptor.[98,99] The main adverse side effects of topiramate use are metabolic acidosis, nephrolithiasis, angle closure glaucoma, acute myopia, and hypertension.[100]

Preclinical studies reported the neuroprotective effect of topiramate in cell cultures and animal models of hypoxic–ischemic injury. The antiexcitotoxic effect of topiramate was demonstrated in cortical cell cultures with oxygen and glucose deprivation (OGD) treated with topiramate (30–300 μM). There was a significant increase in surviving neurons at the higher dose (300 μM) like those cells incubated with a NMDA antagonist.[101] Also was proved the prophylactic treatment of topiramate in a rat model of HIE, demonstrating that intraperitoneal injections of 50 to 100 mg/kg immediately before and after the injury significantly reduced neurological damage by reductions in liquefactive infarction of the hemisphere where the blood flux was limited.[101] Topiramate also demonstrated effective prevention of immature oligodendrocyte loss if administered immediately after the injury (30 mg/kg, intraperitoneal) in a model of periventricular leukomalacia.[102] According to animal studies, the therapeutic frame window for topiramate seems to be $<$2 hours since doses given after this time did not have neuroprotective action.[101] It is important not to exceed the recommended dose and time of topiramate administration since excessive amounts or long-time treatment (10 days) can cause new damage and affect neurocognitive outcomes.[103] In addition, combined therapy of TH and topiramate showed better performance and pathological outcomes.[104]

On the other hand, topiramate has been proved as a combined therapy with TH in HIE newborns. Filippi et al evaluated the pharmacokinetic[105] and safety[106] of topiramate in neonates with HIE. In the first study, 13 newborns with HIE received oral topiramate doses of 5 mg/kg on the first day followed by 3 mg/kg for 2 more days, and children were under deep ($n = 8$) or mild ($n = 5$) hypothermia therapy. Results demonstrated that almost 85% of the children rich serum topiramate concentrations above 5 mg/L after 24 hours of the first dose (reference range: 5–20 mg/L) demonstrating oral absorption maintenance. However,

hypothermia increased topiramate concentrations compared with previous studies on normothermic children, although no adverse side effects related with topiramate use were reported.[105] In a second study ($n = 27$) authors proved the safety of the previous topiramate dose scheme ($n = 11$) and an increased dose of topiramate (5 mg/ kg for 3 days) in combination with deep (30–33°C) and mild hypothermia (32–34°C), no adverse side effects related with topiramate were reported, only was observed a mild and reversible acidosis in newborns under deep hypothermia.[106]

A recent multicenter randomized controlled pilot trial was proved the safety and efficacy of topiramate (10 mg/kg once a day for 3 days) combined with TH versus TH alone. Results showed not statistical differences in death or neurodevelopmental disability after 18 to 24 months of the injury. Although the new dose reached increased serum topiramate concentration of 6.5 to 7 mg/L on the first day of treatment, and an average increase of 12 to 13 mg/L after the third day of treatment. Newborns with topiramate treatment showed a tendency for better seizure control in the treatment group with no adverse side effects.[107]

Nuñez-Ramiro et al also tested the efficacy of topiramate in seizure control, mortality, severity damage, and oxidative stress in neonates with HIE under TH. The topiramate treatment group ($n = 54$) received doses of 5 mg/kg on the first day followed by a maintenance dose of 3 mg/kg/d the subsequent 5 days via nasogastric tube, while placebo group ($n = 52$) received sterile water. The results showed no statistical differences between any of the variables evaluated.[108]

One reason that could explain why topiramate has not shown important neuroprotective and/or antiepileptic effect in neonates with HIE, is due to the low blood concentration reached after oral topiramate administrations of 5 mg/kg dose which hardly enough adequate serum levels between 5 and 20 mg/L to exert an effect.[109] Topiramate can only be administered orally, thus its pharmacokinetic may be affected in hypothermic therapy, due to slower absorption or biotransformation.[105] Marques et al proposed an increase in the dose administered to neonates with HIE to achieve the ideal mean serum concentration (~12 mg/L), starting treatment with the administration of 15 mg/kg/dose on the first day, and a dose of 5 mg/kg on the subsequent 4 days. In addition, it should be considered that once TH is suspended, topiramate clearance is increased by 20.8%.[110] More randomized multicenter clinical trials are needed to establish a standard dose and to prove safety and neuroprotective effects of topiramate in neonates with HIE.

Xenon

Xenon is a noble gas that is employed in medicine as volatile anesthetic.[111] It may participate as a neuroprotective agent against excitotoxicity by the blockade of NMDA, AMPA, and kainite receptors,[112] also by the activation of the two-pore domain potassium channel TREK-1,[113] and the activation of AYP-sensitive potassium channel (K_{ATP}).[114]

Xenon has antiapoptotic action for the activation of B-cell lymphoma 2 proteins (Bcl-2), activation of the cell lymphoma extra-large mitochondrial membrane molecule (Bcl-xL), the downregulation of the apoptosis activator protein (Bax), and preventing the cleavage of caspase-3.[115]

Xenon also preserves neutrophil and monocyte antibacterial capacity and modulates proinflammatory cytokines increasing the activation of nuclear transcription factor kappa B (NF-κB), the production of TNF-α and interleukin 6 (IL-6).[116] These cytokines are involved in the progression of histological damage in the secondary and tertiary phase of the lesion.

Other important neuroprotective effect of xenon treatment after HIE is the upregulation of HIF-1α that upregulates other neuroprotective proteins such as EPO, vascular endothelial growth factor, and glucose transporter 1.[115,117]

Several preclinical HIE studies have demonstrated the benefits of xenon treatment with doses of xenon 30 to 50% for 3 to 5 hours.[118–120] The therapeutic frame window for xenon could be within 5 hours after the injury.[121] In addition, cotreatment of TH and xenon could provide better neuroprotection after HIE (xenon 50% in 30% of oxygen and balanced by nitrogen for 3 hours).[119] However, in severe asphyxia, the cotreatment seems to loss its neuroprotective effect.[122] Besides, in an animal model of antenatal hypoxia, xenon could be safe if administered in subanesthetic dose (35% xenon in 30% oxygen and balanced by nitrogen) to mothers during the childbirth.[120]

The feasibility of xenon was evaluated combined with TH in neonates with moderate-to-severe HIE ($n = 14$). Xenon delivery was started at a median of 11 hours after the injury in a range of 5 to 8 hours, administered at 50% concentration for up to 18 hours. In addition, children were under cooling for 72 hours according to TH protocols. Xenon helped to increase sedation and suppress seizures during the treatment. Three of the 14 children died in the next few days, the rest of the children were evaluated 18 to 20 months later, 7 of the 11 survivors had mental and physical development normal or mildly delayed according to the "Bayley Scales of Infant II," the other 4 children had mental or physical mayor delays. Finally, no adverse effects were reported during or after the xenon therapy demonstrating xenon feasibility for HIE treatment in combination with hypothermia.[123]

Despite the benefits of xenon in the antiexcitotoxicity, its use is limited due to its high costs of manufacturing, implementation of closed-circuit xenon delivery system could be helpful,[124] as reported by Dingley et al who maintained a steady 50% concentrations of xenon with only 0.29 ± 0.19 L/h.[123] Furthermore, there are still doubts regarding the appropriate concentration and time of use, as well as long-term studies of the outcomes after its use, more clinical trials are needed to elucidate these questions.

Neuroregenerative Therapeutics for Hypoxic–Ischemic Encephalopathy

The current treatments for hypoxic–ischemic event are not conclusive in the prevention of neurological outcomes, being urgent to develop corrective, safe and efficacy therapies to restore the function and integrity of the injured tissue. The

new proposal is to develop neuroregenerative therapeutics for those children who already have an important damage, some treatment approaches are steam cell–based therapy, neurotrophic factors, and gonadotropin-releasing hormone (GnRH) agonists.

Mesenchymal Stem Cells–Based Therapy

Mesenchymal stem cells (MSCs) are one of the three multipotent cell types derived from the embryonic stem cells. MSC have the property of self-renewal and differentiate into mesodermal cells such as bone, cartilage, and fat.[125] Nevertheless, their neuroregenerative potential, antiapoptotic, antiexcitotoxic, antioxidant, and immunomodulatory properties have been described.[126] MSC also could induce brain cells proliferation, mainly for paracrine activity through trophic factors liberation such as vascular endothelial growth factor, basic fibroblast growth factor, nerve growth factor, brain-derived neurotrophic factor (BDNF), and liberation of anti-inflammatory cytokines.[127,128]

Preclinical studies with cell cultures and animal models have demonstrated the neuroprotective and neuroregenerative actions of MSC. Wang et al demonstrated the increment of neural stem cells in the subventricular zone and increased neuronal survival in the cortex and CA1 zone of the hippocampus after MSC from human umbilical cord blood treatment in rats who suffer hypoxic–ischemic injury.[129] Hypoxic preconditioning MSC also was related with improved neuroregeneration and function recovery through improved migration of MSC to peri-infarct area, less apoptotic positive cells, and activation of CXCL12, a chemotactic factor, and its receptor CXCR4, producing spontaneous neuronal repair after the MSC transplantation.[130]

On the other hand, Cotten et al evaluated the feasibility and safety of umbilical cord blood cells autologous infusion in a pilot study. A total of 23 newborns with hypoxic–ischemic injury who received TH were included, children were treated with up to four doses of 1 to 5×10^7 cells/kg intravenously, results showed lower mean oxygen saturation after third and fourth cell infusions, but not additional important side effects were reported. Feasibility of MSC from umbilical cord blood also was evaluated, demonstrating that collection, preparation, and infusion of the cells were safe and feasible.[131]

Cotten et al also performed a phase-I open label trial in six neonates >35 weeks' gestational age treated with TH for moderate-to-severe HIE that additionally received allogenic umbilical tissue-derived mesenchymal stromal cells (hCT-MSC). Three children were infused with 2×10^6 cells/kg intravenously in the first 48 postnatal hours and the other three neonates also received a second dose at 2 months. Acute outcomes of infection and side effects were not present demonstrating safety infusion of MSC in children under TH.[132]

Additionally, a phase-I open label clinical trial evaluated the feasibility, safety, and efficacy of Wharton's jelly-derived MSC dose of 10^6 cells/kg that administered twice a month for 2 months by different routes (intrathecally, intramuscularly, and intravenously) in pediatric patients with previous hypoxic–ischemic event; six children were included with age $\leq$12 years, demonstrating a safety profile with mild side effects, such as mild fever, headache, and muscle pain, whit rapid resolution after the first 24 hours of administration.[133]

The advantages of MSC treatment in newborns who suffer HIE are the easy isolation with no ethical concerns because are obtained from discarded neonatal tissues placenta or umbilical cord, reduced risk of immune reaction for autologous transplantation, and avoid the risk of tumor formation unlike embryonic stem cells.[126] However, the encouraging results of more studies are needed for standardization of the safe dose, route of administration, and window of treatment.

Brain-Derived Neurotrophic Factor

BDNF is an important neurotrophin that participates in survival, growth, and synaptic plasticity of nerve cells.[134] There are two types of BDNF, the pro-BDNF and mature BDNF. Each one with higher affinity to a specific receptor, pro-BDNF presents selectivity for the p75 neurotrophin receptor ($p75^{NTR}$) inducing proapoptotic signals. While mature BDNF shows higher affinity for tropomyosin-related kinase receptor type-B (TrkB) activating survival signals by phosphatidylinositol 3-kinase/Akt pathway and the mitogen activated protein kinase/extracellular-signal regulated kinase (MAPK/ERK) pathway.[135] After a hypoxic–ischemic event, the expression of BDNF, its receptors, and the enzymes that participate in the processing of BDNF are increased in the ipsilateral zone of the injury, suggesting the participation of BDNF in the recovery after HIE.[136]

In one in vitro study of human neuroblastoma cells with oxygen and glucose deprivation were subsequently treated with macrophage migration inhibitory factor (MIF), a chemokine that regulates the immune system, demonstrating a protective effect against hypoxia/reperfusion injury due to the increase in BDNF expression. In addition, when the MIF antagonist was administered, the BDNF expression was diminished, and proapoptotic proteins increased.[137] BDNF also exhibit inhibition of neuronal swelling[138] and antiexcitotoxic activity.[139] However, increased levels of BDNF in hippocampus of hypoxic–ischemic rats did not have effect in cognitive impairments after hypoxic–ischemic injury.[140]

The neuroregeneration is another main objective in the treatment of HIE. BDNF has demonstrated to promote neurite regeneration in an in vitro study of axonal ablation in hippocampal neurons by regulating neuronal adhesion and formation of growth cone–like structures or actin waves.[141] In addition, Xue et al studied the effect of BDNF in HIE and showed that BDNF improves the syntaxin1b (Stx1b) expression, a protein that participates in exocytosis of vesicles, also BDNF downregulate the voltage-dependent anion-selective channel protein 1 (VDCA1), proposing both proteins in BDNF neuron survival effect.[138] In an animal model of HIE, the intraventricular administration of BDNF and epidermal growth factor (EGF) exhibited increased number of new neurons in the subventricular zone and striatum, increasing expression of β-III tubulin in the neostriata and better performance on motor test compared with controls.[142]

However, BDNF has some limitations for its regular use. Liu et al described BDNF limitations such as the low-rate transport across the BBB, serum short half-life of just

few minutes, the risky administration route due to its low diffusion rate which would imply BDNF administration directly into the injured area. In addition, BDNF synthesis has expensive manufacturing.[143]

Gonadotropin-Releasing Hormone Agonists

GnRH is a decapeptide produced by hypothalamic neurons and induces the synthesis and secretion pituitary of luteinizing hormone and follicle stimulant hormone, both important hormones for reproduction in mammals.[144] The GnRH has a half-life of less than 10 minutes, due to its short half-life, it is suggested that GnRH receptors are autocrine and paracrine regulators in other tissues.[145] GnRH receptor is member of the G-protein-coupled receptor family and highly express in the adenohypophysis but has also been found in other parts of the central nervous system such as hypothalamus, hippocampus, anterior cingulate cortex, spinal cord, motor cortex, lateral septal nucleus, and the amygdala.[146–148] It has also been found in adrenal tissue and in breast and prostate cancer cells.[149]

The presence of the receptor in other parts of the nervous system may be related with the neurotrophic role of GnRH. Treatment with GnRH or leuprolide acetate (LA), a GnRH agonist, favored the increase of outgrowths, and the length of neurites in studies with cell cultures of cortical neurons and spinal cord from rat embryos.[150,151]

Currently, GnRH agonists are used for the treatment of central precocious puberty, endometriosis, polycystic ovary syndrome, infertility in men, alterations in GnRH secretion, and in the treatment of breast cancer and prostate.[152] Compared with GnRH, the GnRH agonists have a higher affinity for the GnRH receptor and is more resistant to enzymatic degradation and has a powerful effect.[153]

The efficacy of LA in neuroregeneration has been proven in animal models of injured spinal cord, showing improvements in locomotor activity and restoration of urinary dysfunction. In addition, LA demonstrated immunomodulatory action by reducing microglial immunological reaction compared with animals without treatment.[154] By the other hand, a case control study was described the improvements in sensitivity and motor activity in a patient with chronic spinal cord injury after intramuscular administration of 3.75-mg LA, administered monthly for 6 months,[155] and same results were found in a pilot-type phase-II clinical trial after 6 months of the same treatment scheme.[156]

Therefore, GnRH or its agonists could participate in neuroregeneration process after hypoxia–ischemia injury. Chu et al observed decreased expression of GnRH and GnRH receptor and GnRH mRNA at CA1 region of the hippocampus related with apoptosis after a hypoxic–ischemic event.[157] In another study, the prophylactic administration of a GnRH agonist in rats with middle cerebral artery occlusion attenuated the apoptosis of hippocampal neurons after the ischemic-reperfusion injury, evidenced by a smaller number of TUNEL positive pyramidal neurons in CA1 region.[158]

Regarding the immunomodulatory role of GnRH and its agonists, it has been found that in animal models of experimental autoimmune encephalomyelitis, LA reduced the activation of microglia, decreased NF-κB, IL-1β, and IL-17A.[159,160]

Due to its neuroregenerative and immunomodulatory role, GnRH agonist could be a novel treatment for HIE, additional randomized controlled human trials will need to be performed.

Conclusion

HIE has serious repercussions in the neonatal brain due to its greater susceptibility to oxidative damage, the exacerbated immune response, and excitotoxic damage that favors cell death. TH is the standard of care after a hypoxic–ischemic event; however, not all cases are eligible, require special facilities for its implementation and despite its use, many cases have serious neurological outcomes. The development of emerging treatments that aid in the prevention of HIE has been shown to have beneficial effects in some of the injury cascades. However, the results are not always the expected, especially in cases of severe HIE cases in which the use of combined treatments with hypothermia and posterior use of neuroregenerative therapeutics could have better results. Future randomized controlled human trials will need to be performed to determine the standardized dose and duration of the treatments. Also, it is important to analyze the long-term outcomes in the neurological, motor, and cognitive development of children with HIE after any treatment.

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