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Review

Treatment and new progress of neonatal hypoxic-ischemic brain damage

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Summary. Neonatal hypoxic ischemia (HI) results in different extents of brain damage, and immature brain tissue is particularly sensitive to the stimulation of HI. Hypoxic-ischemic brain damage (HIBD) is a common and serious nervous system disease in neonates, for both full-term infants and preterm infants, and is one of the main causes of neonatal death. The surviving infants are often associated with cerebral palsy, mental retardation, and other sequelae, which severely affect quality of life. For term infants, hypoxia and ischemia mainly affect gray matter, whereas in preterm infants, the white matter. However, up to now, inadequate standards and specific measures that can be used to treat hypoxic-ischemic brain injury are available. Recently, in addition to supportive therapy and symptomatic treatment, research on the treatment of hypoxic-ischemic brain injury has focused on the following aspects: hypothermia therapy, stem cell therapy, neuroprotective agents, ibuprofen, and combination therapy. In this review, we will summarize the treatment of HIBD and make suggestions for the future treatment direction.

Key words: Neonate, Hypoxic-ischemic brain damage, Treatment, Hypothermia, Stem cell therapy

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Introduction

Hypoxic-ischemic brain damage (HIBD) can easily occur in infants with complications such as prolonged delivery or prolapse of the umbilical cord (Qureshi et al., 2010). If the injury occurs in full-term newborns, it is called hypoxic-ischemic encephalopathy (HIE) (Volpe, 2001). HIE is a common and serious neurological disease in the neonatal period, which is one of the main causes of neonatal death. Neonatal HIE is a brain injury caused by perinatal asphyxia with an incidence of 0.2-0.4% (Horn et al., 2013). Whereas the incidence in low-birth-weight infants and premature infants is about 60% (Vannucci, 2000), 20-50% of asphyxiated neonates with HIE die in the neonatal period; and up to 25% of survivors will show permanent neuropsychological disorders, such as mental retardation, cerebral palsy, epilepsy, or learning disability (du Plessis and Volpe, 2002; Vannucci and Hagberg, 2004). Neonatal hypoxic ischemia (HI) can trigger the occurrence of sequential cascade neurotoxic events within hours and can last from days to weeks, which results in significant neurological damage (Johnston et al., 2011). In recent years, many studies on the mechanism of HIE and its repair mechanism are found, and the research direction is becoming more and more diversified. However, up to now, few therapies that can be directly utilized in the clinic are available. In recent years, research on HIE treatment has focused on several aspects such as hypothermia therapy (Kida et al., 2013), neuroprotective agent therapy (Nguyen et al., 2014), stem cell therapy (Fang et al., 2013), melatonin, xenon, argon, ibuprofen, and so on. This short review is a brief summary of the recent clinical research and basic research on the

treatment of hypoxic-ischemic brain injury and looks forward to future application prospects.

Therapeutic hypothermia

HIE is a pathological process with multiple links and pathways. In neonatal asphyxia, firstly hypoxic-ischemic injury causes energy metabolism disorder of brain cells, and primary brain cell injury occurs. After resuscitation, brain oxygenation and perfusion recover, resulting in free radical injury, intracellular calcium overload, excitatory amino acids, and other late death processes of nerve cells, that is, delayed brain cell injury after 6-48 hours, which is the secondary energy failure.

No specific treatment for HIE has been found so far. Besides support therapy, hypothermia therapy is still the main clinical measure to improve the prognosis of children with moderate-to-severe HIE no more than 6 hours after birth, including local head cooling, systemic head cooling, and head cooling combined with systemic cooling. No significant difference in the survival rate, side effects, and neuromotor development between selective head cooling and systemic cooling has been found (Celik et al., 2016). The possible mechanisms of mild hypothermia in brain injury treatment include inhibition of metabolic rate, protection of the blood-brain barrier and reduction brain edema (Bonifacio et al., 2012), reduction of diffuse axonal injury, inhibition of apoptosis (Rocha-Ferreira et al., 2018), and inhibition of free radical scavenger consumption and lipid peroxidation (Chevin et al., 2016). Therapeutic hypothermia is a standard treatment for HIE of term or late preterm infants. In the past, it was considered that therapeutic hypothermia could not be used for HIE of preterm infants. However, in recent years, studies have found that hypothermia treatment for HIE of preterm infants seems to be effective. However, risks of mortality and side effects warrant caution in the use of therapeutic hypothermia in preterm infants (Herrera et al., 2018).

Clinical Term or late preterm (≥36w) study

Many multicenter randomized clinical trials (RCTs) have been conducted for hypothermia treatment in children with HIE. In 2008, a multicenter RCT conducted by the National Institute of Child Health and Human Development of the National Institutes of Health showed that systemic hypothermia (33.5°C, 72h) was safe and effective in treating HIE, and could reduce the mortality of children with HIE and the incidence of neurological dysfunction (Shankaran et al., 2008). In 2010, Rutherford et al. reported that hypothermia treatment (anal temperature maintained at 33-34°C for 72h) could improve the prognosis of survivors' nervous system (Rutherford et al., 2010). The results of these two studies suggest that the temperature control maintained between 32°C and 34°C is effective in the treatment of brain injury. Therapy should be started at the earliest. The best treatment effect can be seen within 6h post injury. If it exceeds 6h, the neuroprotective effect of hypothermia may be reduced or disappear (Taylor et al., 2002). Hypothermia treatment should be given for at least 12–24h. If the treatment gets delayed, then the treatment duration should be extended to 72h. The two larger randomized controlled clinical studies mentioned above in the United States and the United Kingdom have 72 h of hypothermia treatment. Edwards et al., analyzed three trials, including 767 infants, and found that moderate hypothermia is associated with a consistent reduction in death and neurological impairment at 18 months in infants with HIE (Edwards et al., 2010). Three years later, Jacobs et al., analyzed 11 randomized controlled trials and found that cooling reduces mortality without causing any increase in the major disability of the survivors (Jacobs et al., 2013). The benefits of cooling on survival and neurodevelopment outweigh the short-term adverse effects. A study identified that compared to the control group, more children in the hypothermia group survived without neurologic abnormalities. Among survivors, children in the hypothermia group had significant reductions in the risk of cerebral palsy and better motor function scores (Azzopardi et al., 2014).

Long-term hypothermia has neuroprotective effects and it increases the incidence of adverse reactions such as coagulation dysfunction and systemic infection. No report of cooling effect was found for more than 72h until the study of Shankaran and his colleague. To some extent, therapeutic hypothermia (72 hours at 33.5°C) for neonatal hypoxic-ischemic encephalopathy reduces death or disability, but the rates continue to be high. Clinicians and researchers have made a new attempt by trying to know whether cooling for more time (120 hours) or to a lower temperature (32.0°C) can reduce death or disability at age 18 months in infants with HIE (Shankaran et al., 2017). The randomized clinical trial found that cooling for longer or lower temperature has no effect on morbidity and mortality of neonates with HIE.

Stem cell transplantation

In recent years, the research of neural stem cell (NSC) transplantation in the treatment of HIE has become increasingly widespread with the rapid development of stem cell technology. NSCs are capable of producing nervous tissue or originating from the nervous system, self-renewal, and producing an NSC and a progeny cell identical to their parents through asymmetric division. To some extent, transplantation of NSC can compensate for the deficiency of endogenous NSCs, replace some degenerated and necrotic neurons because of ischemia and hypoxia, and repair the normal structure and function of the nervous system to a certain extent.

At least two sites of NSCs are present in the central nervous system: the subventricular area-cephalic process-olfactory bulb system and the subgranular cell

layer system in the dentate gyrus of the hippocampus. The subgranular cell zone is the primary germinal area of NSCs similar to the subventricular zone, and NSCs migrate as they develop and slowly enter the cortical granular cell layers to form new neurons (Aly et al., 2015).

In the past decades, NSCs with differentiation potential were obtained from the embryonic central nervous system of mammals, the striatum, hippocampus, subependymal area, and spinal cord of adult individuals. Recently, research work on the mending of nervous system damage, especially the proposal of adult stem cell plasticity, has become a hotspot in regenerative medicine field (Banas et al., 2007), and that makes people focus more on this field. It has been preliminarily confirmed that non-nervous system-derived adult stem cells, such as hematopoietic stem cells and mesenchymal stem cells, can discriminate into nerve cells (Nagai et al., 2007) in vivo and in vitro and can mend the damaged nervous system to a certain extent.

Animal study

It has been demonstrated that mesenchymal stem cells can successfully penetrate the blood-brain barrier by intraperitoneal injection into the HIE animal model, and the barriers might also improve the function of the damaged nervous system (Cho et al., 2006). It has been found that umbilical cord mesenchymal stem cell exosomes can ease perinatal brain injury induced by microglia-mediated neuroinflammation (Thomi et al., 2019). Some studies have suggested that umbilical cord mesenchymal stem cells and cord blood monocytes can advance the memory of neonatal rats after HI (Zhang et al., 2019). Through animal studies, it has been demonstrated that the intracardiac injection of dental pulp stem cells after neonatal HI can prevent cognitive impairment in rats (Sanches et al., 2018). A study by Li et al. suggested that umbilical cord-derived mesenchymal stem cells play a neuroprotective role in hypoxic-ischemic injury by reducing the inflammatory response and inhibiting apoptosis (Li et al., 2020). Stem cell therapy offers the potential to replace the damaged cells during and after hypoxia-ischemia insult and to enhance the auto-regeneration process (Douglas et al., 2012).

Erythropoietin

It was found that erythropoietin receptor (Epo-R) was mainly expressed in bone marrow hematopoietic stem cells, neurons, glial cells, and vascular endothelial cells in the brain tissue. Through animal experiments, it has been found that EPO was an effective neuroprotective agent in the occurrence of hypoxic-ischemic brain injury (Fan et al., 2011).

Animal study

A study showed that neonatal erythropoietin reduced the abnormalities in gait, social interaction, and diffusion tensor imaging in rats with prenatal brain injury (Robinson et al., 2018). The single dose of EPO at 5000 μ/kg immediately or 48 h after HI injury could significantly improve the recovery of P2 rats, and no adverse reactions were found in either of the EPO treatments (Ren et al., 2017). EPO significantly improved the neurobehavioral performance before and after the treatment and protected them from HI-induced neuronal death, microglia activation, and loss of mature oligodendrocytes and hippocampal neurons (Lan et al., 2016). The long-term protective effect of EPO on the HI model in neonatal rats suggests that neurotrophic activity in the brain may be an effective way to treat hypoxic-ischemic brain injury.

Clinical study

Furthermore, clinical studies also demonstrate that EPO has a good effect on the treatment of full-term neonates with perinatal asphyxia. Neurons and astrocytes in the central nervous system can secrete EPO by the paracrine or autocrine way and bind to EPO receptors on the membrane of nearby neurons. Mulkey et al. found that 9/20 erythropoietin-treated vs 12/24 placebo-treated infants with HIE had acute brain injury (Mulkey et al., 2017). In infants with acute brain injury, the amount of damage in the erythropoietin group was lower than that in the placebo group. The greater the damage, the lower the neurodevelopmental score at 12 months. Malla et al. showed that 40% of the newborns in the treatment group died or had moderate or severe disabilities and 70% in the placebo group (Malla et al., 2017). Survivors in the treatment group had a lower risk of cerebral palsy, and fewer infants were assessed with anticonvulsants. Neonatal brain magnetic resonance imaging demonstrated more abnormalities in the placebo group. Other neurological outcomes did not improve significantly. It has been concluded that EPO alone could decrease the risk of death or disability in full-term newborns with moderate or severe encephalopathy.

Melatonin

Melatonin is a neuroendocrine hormone which is mainly synthesized by the pineal body and easily passes through blood-brain barrier and blood-placenta barrier. Its biological functions include regulating the sleep cycle, antioxidation, anti-inflammatory, regulating lipid, and glucose metabolism. In recent years, research at home and abroad suggested that melatonin plays a neuroprotective role in neonatal brain injury induced by HI.

Animal study

In 2012, a study by Balduini et al. using a rat model of HIBD demonstrated that melatonin could reduce oxidative stress, inflammatory cell recruitment, and

activation of glial cells in the cerebral cortex after neonatal HI insult (Balduini et al., 2012). Carloni et al. demonstrated that in rats, melatonin could reduce the endoplasmic reticulum (ER) stress induced by neonatal HI (Carloni et al., 2014). In 2017, Blanco and his colleagues demonstrated that early in the first steps of the ischemic cascade, melatonin could function and protect the brain from hypoxic/ischemic-derived damage by influencing the NO/NOS pathway and reducing the oxidative and nitrosative stress (Blanco et al., 2017).

Clinical study

A prospective trial in 2015 found that compared to hypothermia group, the melatonin/hypothermia group had fewer seizures and less white matter abnormalities. Later at 6 months, the melatonin/hypothermia group had improved the survival rate without developmental or neurological abnormalities. These results indicated that early administration of melatonin to asphyxiated term neonates was feasible and might ameliorate brain injury (Aly et al., 2015). A randomized control trial conducted from 2016 to 2017 found that melatonin (10mg orally) as an adjunct therapy in the management of newborns with HIE led to improved survival rate (Ahmad et al., 2018). Balduini W et al. aimed to evaluate melatonin safety, pharmacokinetics (PK), and dosage in neonates with HIE undergoing hypothermia and adjunctive therapy with melatonin (Balduini et al., 2019). The results showed that half-life and clearance of melatonin were prolonged. Hypothermia did not affect melatonin PK.

Magnesium sulfate

Magnesium ion is an important cation in the human body that plays an important role in phosphorylation, ATP formation, and activation of sodium-potassium ATPase. To play a role in cell protection, it reduces cell edema, reduces the permeability of membrane, and protects cell membrane integrity. In many processes of HIE, intracellular calcium overload is considered to be the last common pathway of cell death, including necrosis and apoptosis. Because of the nonspecific antagonistic effect of magnesium on the calcium channel and the noncompetitive antagonistic effect of NMDA receptor, it is expected to achieve the protective effect by blocking calcium overload on hypoxic-ischemic brain injury.

Animal study

Research indicated that $MgSO_4$ could provide marked preconditioning protection both in rat and in mice HIE model (Koning et al., 2018). In rat pups, pretreatment with $MgSO_4$ attenuates white matter damage by preventing the cell death of pre-OL (Itoh et al., 2016; Seyama et al., 2018) and might have a role in preventing neuronal apoptosis because of neonatal hypoxic-ischemic brain injury (Türkyilmaz et al., 2002). These data might support the use of magnesium sulfate in the clinical setting. In sheep models, $MgSO_4$ may be helpful in the treatment of perinatal epilepsy, and the therapeutic effect is related to gender (Bennet et al., 2018).

Clinical study

Douglas et al. demonstrated that magnesium sulfate could decrease the risk of eclampsia with no substantive harmful effects to mother or baby in the short term (Douglas et al., 2002). Immediately before very preterm birth, magnesium sulfate given to women might improve neonatal outcomes with no serious harmful effects. (Crowther et al., 2003; Marret et al., 2007, 2008). In 2008, a randomized controlled trial conducted by Dwight J Rouse group showed that before anticipated early preterm delivery, fetal exposure to magnesium sulfate could reduce the rate of cerebral palsy among survivors (Rouse et al., 2008). Ichiba et al. found that postnatal $MgSO_4$ infusion (250 mg/kg per day) for 3 days is safe and can improve short-term outcomes in infants with severe birth asphyxia (Ichiba et al., 2002).

Xenon

Xenon is the most stable inert gas that has no toxicity or carcinogenicity. It does not undergo biotransformation in the body but is exhaled in the original form through the lung after inhalation. In newborn animals, ammonia gas with a concentration lower than anesthesia has significant neuroprotective properties.

Animal study

Many animal models, such as HIE in rats and pigs, have been used to study the mechanism of xenon. Studies provided evidence for xenon's preconditioning effect, which promotes survival against neuronal injury (Ma et al., 2006; Luo et al., 2008). A team has conducted research from the perspective of impaired automatic regulation of cerebral perfusion during hypoxia and ischemia. They demonstrated that independent of the insult severity, Xenon abolished the secondary cerebrovascular pressure reactivity (PRx) peak, increased mean arterial blood pressure and cerebral perfusion pressure, and preserved the PRx (Chakkarapani et al., 2013). Yin et al. found that xenon could decrease neuronal apoptosis via Bcl-2 and CLIC4-mediated pathways. They also found that the therapeutic time window of xenon could be extended for up to 5 h (Yin et al., 2018). Koziakova et al. found that xenon's neuroprotective effect might be mediated by the inhibition of the N-methyl-d-aspartate receptor at the glycine site (Koziakova et al., 2019).

Clinical study

A few clinical studies have been found on xenon in

HIE treatment. A clinical study by Dingley J demonstrated that combining 50% xenon for up to 18 hours with 72 hours of cooling was feasible, with no adverse effects seen with 18 months of follow-up (Dingley et al., 2014).

Whether xenon combined with therapeutic hypothermia is better or not, the conclusions of various studies are not consistent. Some researchers might consider that it is better, and some consider that xenon treatment does not enhance the effect of hypothermia treatment. In animals, a study by Hobbs et al. demonstrated that the xenon/hypothermia combination confers better protection after HI than either treatment alone. The functional improvement is almost complete and is accompanied by greatly improved histopathology (Hobbs et al., 2008). While in 2011, Faulkner S and colleagues found that compared with hypothermia, xenon-augmented hypothermia did not reach statistical significance for any measure. In clinical study, Azzopardi et al found that the administration of xenon within the delayed timeframe used in this trial is feasible and safe, but xenon did not enhance the neuroprotective effect of cooling therapy after birth (Azzopardi et al., 2016).

Argon

Xenon has a good neuroprotective effect, but its disadvantage lies in its high price and high requirements for transportation equipment. Argon is an inert gas widely used in the industry. Its cost is far lower than xenon. In animals, using a model of hypoxic-ischemic brain injury and neuronal culture model, researchers found the neuroprotective effect of argon is almost the same as that of xenon (Jawad et al., 2009). While Xenon and argon are equally effective as neuroprotectants against hypoxia–ischemia in vitro, with both gases preventing injury development, they might go through different pathways (Koziakova et al., 2019). Argon (inhaled 45-50%) combined with augmented hypothermic protection at 48h after hypoxia–ischemia demonstrated faster EEG recovery, improved brain energy metabolism, and reduced cell death. Argon may provide an inexpensive and practical therapy to augment cooling for neonatal encephalopathy in the future (Broad et al., 2016).

Ibuprofen

Ibuprofen treatment after hypoxic-ischemic brain injury might also be an effective drug intervention, which limits the effects of neuroinflammatory mediators (Wixey et al., 2012). It is a widely used nonsteroidal anti-inflammatory drug, which has antipyretic, analgesic, and anti-inflammatory effects. Ibuprofen is also commonly used in newborns for the treatment of patent ductus arteriosus (Varvarigou et al., 1996). The molecular mechanism of ibuprofen action demonstrates that it can inhibit the activity of cyclooxygenase (COX-1 and COX-2).

Animal study

The pathogenesis of ischemic brain injury in the neonatal HI model involves the increase in COX-2 expression, and the increase in COX-2 activity will cause central nervous system injury through oxidative stress or the synthesis of neurotoxic prostaglandins (PGA1 and PGE1). By reducing ischemic brain damage in adult rats, infarct size (Park et al., 2005), and neonatal HI white matter damage, ibuprofen can inhibit COX-2. Therefore, some studies have demonstrated that NSAIDs have brain-protective effects under cerebral ischemia (Wallenquist et al., 2012). Moreover, the anti-inflammatory effect of ibuprofen may be because of its inhibition of microglial activation (Lim et al., 2000) and its influence on the production of pro-inflammatory cytokines in the brain.

Perspective and challenges

At present, therapeutic hypothermia is one of the standard treatments for HIE, but there are still some problems. There are certain conditions for the application of therapeutic hypothermia--hypothermia should be administered in term and late preterm infants with moderate-to-severe hypoxic ischemic encephalopathy if identified before six hours of age. Therapeutic hypothermia does not reduce the mortality of severe HIE. It may be due to systemic inflammatory response after rewarming, which is contrary to the neuroprotective effect of HT (Rocha-Ferreira et al., 2017). Further trials to determine the appropriate techniques of cooling, including refinement of patient selection, duration of cooling, and method of providing therapeutic hypothermia, will refine the understanding of this intervention.

The treatment of hypoxic-ischemic brain injury requires the symptomatic supportive treatment and deep-seated treatment for the etiology. The current research in this field is not in-depth. The choice of time for moderate hypothermia treatment is critical. Long-term mild hypothermia has a neuroprotective effect, but it increases the risk of infection.

Although stem cell therapy has significant potential in HIE treatment, still many concerns need to be addressed, such as 1. the time, method, location, and quantity of NSC transplantation need further study and confirmation, 2. directional differentiation and rapid proliferation of NSCs, and 3. tumor formation after the transplantation of NSCs. Therefore, the treatment of hypoxic-ischemic brain injury with NSCs needs to be further improved.

In recent years, many animal and clinical studies are found on the combination of two therapies like mild hypothermia and adjuvant therapy or magnesium sulfate

and melatonin combined therapy.

Animal study

The results suggested that these agents might confer a benefit in the treatment of infants with HIE (Cetinkaya et al., 2011). Compared with HT, the small benefits of Mg + HT are unlikely to translate into substantial long-term improvement.

Clinical study

Filippi et al. demonstrated that topiramate combined with hypothermia had no significant difference in safety and primary and secondary outcomes compared with simple hypothermia for HIE, but the combined treatment group could minimize the incidence of epilepsy (Filippi et al., 2018). Aly et al. demonstrated that the combination of melatonin and systemic hypothermia in HIE treatment can achieve better prognostic results-that is, fewer epileptic seizures during follow-up electroencephalogram examinations and lesser white matter lesions in magnetic resonance imaging examinations (Aly et al., 2015). Combined magnesium sulfate with melatonin, two agents acting at different stages of HI brain damage, significantly reduced the percent of infarcted brain volume and TUNEL positivity. This also shows that further study on the combination of multiple therapies is needed in the future (Lingam et al., 2019).

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References

Ahmad Q.M., Chishti A.L. and Waseem N. (2018). Role of melatonin in management of hypoxic ischaemic encephalopathy in newborns: A randomized control trial. J. Pak. Med. Assoc. 68, 1233-1237.

Aly H., Elmahdy H., El-Dib M., Rowisha M., Awny M., El-Gohary T., Elbatch M., Hamisa M. and El-Mashad A.R. (2015). Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study. J. Perinatol. 35, 186-191.

Azzopardi D., Strohm B., Marlow N., Brocklehurst P., Deierl A., Eddama O., Goodwin J., Halliday H.L., Juszczak E., Kapellou O., Levene M., Linsell L., Omar O., Thoresen M., Tusor N., Whitelaw A., Edwards A.D. and TOBY Study Group (2014). Effects of hypothermia for perinatal asphyxia on childhood outcomes. N. Engl. J. Med. 371, 140-149.

Azzopardi D., Robertson N.J., Bainbridge A., Cady E., Charles-Edwards G., Deierl A., Fagiolo G., Franks N.P., Griffiths J., Hajnal J., Juszczak E., Kapetanakis B., Linsell L., Maze M., Omar O., Strohm B., Tusor N. and Edwards A.D. (2016). Moderate hypothermia within 6 h of birth plus inhaled xenon versus moderate hypothermia alone after birth asphyxia (TOBY-Xe): a proof-of-concept, open-label, randomised controlled trial. Lancet Neurol. 15, 145-153.

Balduini W., Carloni S., Perrone S., Bertrando S., Tataranno M.L., Negro S., Proietti F., Longini M. and Buonocore G. (2012). The use of melatonin in hypoxic-ischemic brain damage: an experimental study. J. Matern. Fetal. Neonatal Med. Suppl. 1, 119-124.

Balduini W., Weiss MD., Carloni S., Rocchi M., Sura L., Rossignol C., Longini M., Bazzini F., Perrone S., Ott D., Wadhawan R. and Buonocore G. (2019). Melatonin pharmacokinetics and dose extrapolation after enteral infusion in neonates subjected to hypothermia. J. Pineal. Res. 66, e12565.

Banas A., Yamamoto Y., Teratani T. and Ochiya T. (2007). Stem cell plasticity: Learning from hepatogenic differentiation strategies. Dev. Dyn. 236, 3228-3241.

Bennet L., Galinsky R., Draghi V., Lear C.A., Davidson J.O., Unsworth C.P. and Gunn A.J. (2018). Time and sex dependent effects of magnesium sulphate on post-asphyxial seizures in preterm fetal sheep. J. Physiol. 596, 6079-6092.

Blanco S., Hernández R., Franchelli G., Ramos-Álvarez M.M. and Peinado M.Á. (2017). Melatonin influences NO/NOS pathway and reduces oxidative and nitrosative stress in a model of hypoxic-ischemic brain damage. Nitric Oxide 62, 32-43.

Bonifacio S.L., Saporta A., Glass H.C., Lee P., Glidden D.V., Ferriero D.M., Barkovich A.J. and Xu D. (2012). Therapeutic hypothermia for neonatal encephalopathy results in improved microstructure and metabolism in the deep gray nuclei. AJNR. Am. J. Neuroradiol. 33, 2050-2055.

Broad K.D., Fierens I., Fleiss B., Rocha-Ferreira E., Ezzati M., Hassell J., Alonso-Alconada D., Bainbridge A., Kawano G., Ma D., Tachtsidis I., Gressens P., Golay X., Sanders R.D. and Robertson N.J. (2016). Inhaled 45-50% argon augments hypothermic brain protection in a piglet model of perinatal asphyxia. Neurobiol. Dis. 87, 29-38.

Carloni S., Albertini M.C., Galluzzi L., Buonocore G., Proietti F. and Balduini W. (2014). Melatonin reduces endoplasmic reticulum stress and preserves sirtuin 1 expression in neuronal cells of newborn rats after hypoxia-ischemia. J. Pineal. Res. 57, 192-199.

Celik Y., Atıcı A., Gulası S., Okuyaz C., Makharoblıdze K. and Sungur M.A. (2016). Comparison of selective head cooling versus whole-body cooling. Pediatr. Int. 58, 27-33.

Cetinkaya M., Alkan T., Ozyener F., Kafa I.M., Kurt M.A. and Koksal N. (2011). Possible neuroprotective effects of magnesium sulfate and melatonin as both pre- and post-treatment in a neonatal hypoxic-ischemic rat model. Neonatology 99, 302-310.

Chakkarapani E., Dingley J., Aquilina K., Osredkar D., Liu X. and Thoresen M. (2013). Effects of xenon and hypothermia on cerebrovascular pressure reactivity in newborn global hypoxic-ischemic pig model. J. Cereb. Blood Flow Metab. 33, 1752-1760.

Chevin M., Guiraut C., Maurice-Gelinas C., Deslauriers J., Grignon S. and Sébire G. (2016). Neuroprotective effects of hypothermia in inflammatory-sensitized hypoxic-ischemic encephalopathy. Int. J. Dev. Neurosci. 55, 1-8.

Cho Y.H., Kim H.S., Lee K.H., Lee Y.E. and Chang J.W. (2006). The behavioral effect of human mesenchymal stem cell transplantation in cold brain injured rats. Acta. Neurochir. Suppl. 99, 125-132.

Crowther C.A., Hiller J.E., Doyle L.W., Haslam R.R. and Australasian Collaborative Trial of Magnesium Sulphate (ACTOMg SO4) Collaborative Group (2003). Effect of magnesium sulfate given for neuroprotection before preterm birth: a randomized controlled trial. JAMA 290, 2669-2676.

Dingley J., Tooley J., Liu X., Scull-Brown E., Elstad M., Chakkarapani E., Sabir H. and Thoresen M. (2014). Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. Pediatrics 13, 809-818.

Douglas-Escobar M., Rossignol C., Steindler D., Zheng T. and Weiss M.D. (2012). Neurotrophin-induced migration and neuronal differentiation of multipotent astrocytic stem cells in vitro. PLoS One 7, e51706.

du Plessis A.J. and Volpe J.J. (2002). Perinatal brain injury in the preterm and term newborn. Curr. Opin. Neurol. 15, 151-157.

Edwards A.D., Brocklehurst P., Gunn A.J., Halliday H., Juszczak E., Levene M., Strohm B., Thoresen M., Whitelaw A. and Azzopardi D. (2010). Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. BMJ 340, c363.

Fan X., Heijnen C.J., van der Kooij M.A., Groenendaal F. and van Bel F. (2011). Beneficial effect of erythropoietin on sensorimotor function and white matter after hypoxia-ischemia in neonatal mice. Pediatr. Res. 69, 56-61.

Fang C.Z., Yang Y.J., Wang Q.H., Yao Y., Zhang X.Y. and He X.H. (2013). Intraventricular injection of human dental pulp stem cells improves hypoxic-ischemic brain damage in neonatal rats. PLoS One 8, e66748.

Filippi L., Fiorini P., Catarzi S., Berti E., Padrini L., Landucci E., Donzelli G., Bartalena L., Fiorentini E., Boldrini A., Giampietri M., Scaramuzzo R.T., la Marca G., Della Bona M.L., Fiori S., Tinelli F., Bancale A., Guzzetta A., Cioni G., Pisano T., Falchi M. and Guerrini R. (2018). Safety and efficacy of topiramate in neonates with hypoxic ischemic encephalopathy treated with hypothermia (NeoNATI): a feasibility study. J Matern Fetal. Neonatal Med. 31, 973-980.

Herrera T.I., Edwards L., Malcolm W.F., Smith P.B., Fisher K.A., Pizoli C., Gustafson K.E., Goldstein R.F., Cotten C.M., Goldberg R.N. and Bidegain M. (2018). Outcomes of preterm infants treated with hypothermia for hypoxic-ischemic encephalopathy. Early Hum. Dev.125, 1-7.

Hobbs C., Thoresen M., Tucker A., Aquilina K., Chakkarapani E. and Dingley J. (2008). Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. Stroke 39, 1307-1313.

Horn A.R., Swingler G.H., Myer L., Harrison M.C., Linley L.L., Nelson C., Tooke L., Rhoda N.R. and Robertson N.J. (2013). Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. J. Perinat. Med. 41, 211-217.

Ichiba H., Tamai H., Negishi H., Ueda T., Kim T.J., Sumida Y., Takahashi Y., Fujinaga H., Minami H. and Kansai Magnesium Study Group (2002). Randomized controlled trial of magnesium sulfate infusion for severe birth asphyxia. Pediatr. Int. 44, 505-509.

Itoh K., Maki T., Shindo A., Egawa N., Liang A.C., Itoh N., Lo E.H., Lok J. and Arai K. (2016). Magnesium sulfate protects oligodendrocyte lineage cells in a rat cell-culture model of hypoxic-ischemic injury. Neurosci. Res. 106, 66-69.

Jacobs S.E., Berg M., Hunt R., Tarnow-Mordi W.O., Inder T.E. and Davis P.G. (2013). Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst. Rev. 1, CD003311.

Jawad N., Rizvi M., Gu J., Adeyi O., Tao G., Maze M. and Ma D. (2009). Neuroprotection (and lack of neuroprotection) afforded by a series of noble gases in an in vitro model of neuronal injury. Neurosci. Lett. 460, 232-236.

Johnston M.V., Fatemi A., Wilson M.A and Northington F. (2011). Treatment advances in neonatal neuroprotection and neurointensive care. Lancet Neurol. 10, 372-382.

Kida H., Nomura S., Shinoyama M., Ideguchi M., Owada Y. and Suzuki M. (2013). The effect of hypothermia therapy on cortical laminar disruption following ischemic injury in neonatal mice. PLoS One. 8, e68877.

Koning G., Lyngfelt E., Svedin P., Leverin AL., Jinnai M., Gressens P., Thornton C., Wang X., Mallard C. and Hagberg H. (2018). Magnesium sulphate induces preconditioning in preterm rodent models of cerebral hypoxia-ischemia. Int. J. Dev. Neurosci. 70, 56-66.

Koziakova M., Harris K., Edge C.J., Franks N.P., White I.L. and Dickinson R. (2019). Noble gas neuroprotection: xenon and argon protect against hypoxic-ischaemic injury in rat hippocampus in vitro via distinct mechanisms. Br. J. Anaesth. 123, 601-609.

Lan K.M., Tien L.T., Cai Z., Lin S., Pang Y., Tanaka S., Rhodes P.G., Bhatt A.J., Savich R.D. and Fan L.W. (2016). Erythropoietin ameliorates neonatal hypoxia-ischemia-induced neurobehavioral deficits, neuroinflammation, and hippocampal injury in the juvenile rat. Int. J. Mol. Sci. 17, 289.

Li F., Zhang K., Liu H., Yang T., Xiao D.J. and Wang Y.S. (2020). The neuroprotective effect of mesenchymal stem cells is mediated through inhibition of apoptosis in hypoxic ischemic injury. World. J. Pediatr. 16, 193-200.

Lim G.P., Yang F., Chu T., Chen P., Beech W., Teter B., Tran T., Ubeda O., Ashe K.H., Frautschy S.A. and Cole G.M. (2000). Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J. Neurosci. 20, 5709-5714.

Lingam I., Meehan C., Avdic-Belltheus A., Martinello K., Hristova M., Kaynezhad P., Bauer C., Tachtsidis I., Golay X. and Robertson N.J. (2019). Short-term effects of early initiation of magnesium infusion combined with cooling after hypoxia-ischemia in term piglets. Pediatr. Res. 86, 699-708.

Luo Y., Ma D., Ieong E., Sanders RD., Yu B., Hossain M. and Maze M. (2008). Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology 109, 782-789.

Ma D., Hossain M., Pettet G.K., Luo Y., Lim T., Akimov S., Sanders R.D., Franks N.P. and Maze M. (2006). Xenon preconditioning reduces brain damage from neonatal asphyxia in rats. J. Cereb. Blood Flow Metab. 26, 199-208.

Malla R.R., Asimi R., Teli M.A., Shaheen F. and Bhat M.A. (2017). Erythropoietin monotherapy in perinatal asphyxia with moderate to severe encephalopathy: a randomized placebo-controlled trial. J. Perinatol. 37, 596-601.

Marret S., Marpeau L., Zupan-Simunek V., Eurin D., Lévêque C., Hellot M-F., Bénichou J. and PREMAG trial group. (2007). Magnesium sulphate given before very-preterm birth to protect infant brain: the randomised controlled PREMAG trial. BJOG 114, 310-318.

Marret S., Marpeau L. and Bénichou J. (2008). Benefit of magnesium sulfate given before very preterm birth to protect infant brain. Pediatrics 121, 225-226.

Mulkey S.B., Ramakrishnaiah R.H., McKinstry R.C., Chang T., Mathur A.M., Mayock D.E., Van Meurs K.P., Schaefer G.B., Luo C., Bai S., Juul S.E. and Wu Y.W. (2017). Erythropoietin and brain magnetic resonance imaging findings in hypoxic-ischemic encephalopathy: Volume of acute brain injury and 1-year neurodevelopmental outcome. J. Pediatr. 186, 196-199.

Nagai A., Kim W.K., Lee H.J., Jeong H.S., Kim K.S., Hong S.H., Park

I.H. and Kim S.U. (2007). Multilineage potential of stable human mesenchymal stem cell line derived from fetal marrow. PLoS One 2, e1272.

Nguyen A.Q., Cherry B.H., Scott G.F., Ryou M.G. and Mallet R.T. (2014). Erythropoietin: Powerful protection of ischemic and post-ischemic brain. Exp. Biol. Med. (Maywood) 239,1461-1475.

Park E.M., Cho B.P., Volpe B.T., Cruz M.O., Joh T.H. and Cho S. (2005). Ibuprofen protects ischemia-induced neuronal injury via upregulating interleukin-1 receptor antagonist expression. Neuroscience 132, 625-631.

Qureshi A.M., ur Rehman A. and Siddiqi T.S. (2010). Hypoxic ischemic encephalopathy in neonates. J. Ayub. Med. Coll. Abbottabad 22,190-193.

Ren Q., Jiang Z.H., Zhang X.F and Yang Q.Z. (2017). Effects of erythropoietin on neonatal hypoxia-ischemia brain injury in rat model. Physiol. Behav. 169, 74-81.

Robinson S., Corbett C.J., Winer J.L., Chan L.A.S., Maxwell J.R., Anstine C.V., Yellowhair T.R., Andrews N.A., Yang Y., Sillerud L.O. and Jantzie L.L. (2018). Neonatal erythropoietin mitigates impaired gait, social interaction and diffusion tensor imaging abnormalities in a rat model of prenatal brain injury. Exp. Neurol. 302, 1-13.

Rocha-Ferreira E., Kelen D., Faulkner S., Broad K.D., Chandrasekaran M., Kerenyi Á., Kato T., Bainbridge A., Golay X., Sullivan M., Kramer B.W. and Robertson N.J. (2017). Systemic pro-inflammatory cytokine status following therapeutic hypothermia in a piglet hypoxia-ischemia model. J. Neuroinflammation. 14, 44.

Rocha-Ferreira E., Vincent A, Bright S., Peebles D.M. and Hristova M. (2018). The duration of hypothermia affects short-term neuroprotection in a mouse model of neonatal hypoxic ischaemic injury. PLoS One 13, e0199890.

Rouse D.J., Hirtz D.G., Thom E., Varner M.W., Spong C.Y., Mercer B.M., Iams J.D., Wapner R. J., Sorokin Y., Alexander J.M., Harper M., Thorp Jr J.M., Ramin S.M., Malone F.D., Carpenter M., Miodovnik M., Moawad A., O'Sullivan M.J., Peaceman A.M., Hankins G.DV., Langer O., Caritis S.N., Roberts J.M. and Eunice Kennedy Shriver NICHD Maternal-Fetal Medicine Units Network. (2008). A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy. N. Engl. J. Med. 359, 895-905.

Rutherford M., Ramenghi L.A., Edwards A.D., Brocklehurst P., Halliday H., Levene M., Strohm B., Thoresen M., Whitelaw A. and Azzopardi D. (2010). Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. Lancet Neurol. 9, 39-45.

Sanches E.F., Valentim L., de Almeida Sassi F., Bernardi L., Arteni N., Weis S.N., Odorcyk F.K., Pranke P. and Netto C.A. (2018). Intracardiac injection of dental pulp stem cells after neonatal hypoxia-ischemia prevents cognitive deficits in rats. Neurochem. Res. 43, 2268-2276.

Seyama T., Kamei Y., Iriyama T., Imada S., Ichinose M., Toshimitsu M., Fujii T. and Asou H. (2018). Pretreatment with magnesium sulfate attenuates white matter damage by preventing cell death of developing oligodendrocytes. J. Obstet. Gynaecol. Res. 44, 601-607.

Shankaran S., Pappas A., Laptook A.R., McDonald S.A., Ehrenkranz R.A, Tyson J.E., Walsh M., Goldberg R.N., Higgins R.D., Das A. and NICHD Neonatal Research Network (2008). Outcomes of safety and effectiveness in a multicenter randomized, controlled trial of whole-body hypothermia for neonatal hypoxic-ischemic encephalopathy. Pediatrics122, e791-798.

Shankaran S., Laptook A.R., Pappas A., McDonald S.A., Das A., Tyson J.E., Poindexter B.B., Schibler K., Bell E.F., Heyne R.J., Pedroza C., Bara R., Van Meurs K.P., Huitema C.M.P., Grisby C., Devaskar U., Ehrenkranz R.A., Harmon H.M., Chalak L.F., DeMauro S.B., Garg M., Hartley-McAndrew M.E., Khan A.M., Walsh M.C., Ambalavanan N., Brumbaugh J.E., Watterberg K.L., Shepherd E.G., Hamrick S.E.G., Barks J., Cotten C.M., Kilbride H.W., Higgins R.D. and Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. (2017). Effect of depth and duration of cooling on death or disability at age 18 months among neonates with hypoxic-ischemic encephalopathy: A randomized clinical trial. JAMA 318, 57-67.

Taylor D.L., Mehmet H., Cady E.B. and Edwards A.D. (2002). Improved neuroprotection with hypothermia delayed by 6 hours following cerebral hypoxiaischemia in the 14-day-old rat. Pediatr. Res. 51, 13-19.

Thomi G., Surbek D., Haesler V., Joerger-Messerli M. and Schoeberlein A. (2019). Exosomes derived from umbilical cord mesenchymal stem cells reduce microglia-mediated neuroinflammation in perinatal brain injury. Stem Cell Res. Ther. 10, 105.

Türkyilmaz C., Türkyilmaz Z., Atalay Y., Söylemezoglu F. and Celasun B. (2002). Magnesium pre-treatment reduces neuronal apoptosis in newborn rats in hypoxia-ischemia. Brain Res. 955, 133-137.

Vannucci R.C. (2000). Hypoxic-ischemic encephalopathy. Am. J. Perinatol. 17, 113-120.

Vannucci S.J. and Hagberg H. (2004). Hypoxia-ischemia in the immature brain. J. Exp. Biol. 207, 3149-3154.

Varvarigou A., Bardin C.L., Beharry K., Chemtob S., Papageorgiou A. and Aranda J.V. (1996). Early ibuprofen administration to prevent patent ductus arteriosus in premature newborn infants. JAMA 275, 539-544.

Volpe J.J. (2001). Perinatal brain injury: From pathogenesis to neuroprotection. Ment. Retard. Dev. Disabil. Res. Rev. 7, 56-64.

Wallenquist U., Holmqvist K., Hånell A., Marklund N., Hillered L. and Forsberg-Nilsson K. (2012). Ibuprofen attenuates the inflammatory response and allows formation of migratory neuroblasts from grafted stem cells after traumatic brain injury. Restor. Neurol. Neurosci. 30, 9-19.

Wixey J.A., Reinebrant H.E. and Buller K.M. (2012). Post-insult ibuprofen treatment attenuates damage to the serotonergic system after hypoxia-ischemia in the immature rat brain. J. Neuropathol. Exp. Neurol. 27, 1137-1148.

Yin X., Zhao J., Jiang H., Li L., Jiang J., Xi H., Peng X., Yin X., Shi X. and Zhang L. (2018). Impact of xenon on CLIC4 and Bcl-2 expression in lipopolysaccharide and hypoxia-ischemia-induced periventricular white matter damage. Neonatology 113, 339-346.

Zhang J., Yang C., Chen J., Luo M., Qu Y., Mu D. and Chen Q. (2019). Umbilical cord mesenchymal stem cells and umbilical cord blood mononuclear cells improve neonatal rat memory after hypoxia-ischemia. Behav. Brain. Res. 362, 56-63.

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