

Novel Horizons in Neuroprotection of the Newborn: Cooling is Just the Beginning

Manigandan Chandrasekaran¹ and Kishore Kumar^{2*}

¹Consultant Neonatologist, Cloudnine Hospital, Chennai, India

²Consultant Neonatologist, Cloudnine Hospital, Bengaluru, India

***Corresponding Author:** Prof. Dr. R. Kishore Kumar, Consultant Neonatologist and Paediatrician, Cloudnine Hospital, 1533, 9th Main, 3rd Block Jayanagar, Bengaluru, 560011, India.

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Abstract

Hypoxic ischemic encephalopathy (HIE) is a major cause of neonatal mortality and morbidity. Of those affected neonates, 15-20% of them will die in the postnatal period, and an additional 25% will sustain childhood disabilities. However, recent studies have shown that treatment with moderate hypothermia for HIE increases survival without neurologic deficit at the age of 18 months, providing us a proof that neuroprotection of the newborn is possible. Despite the beneficial effects of hypothermia, approximately half the infants who receive hypothermia still have abnormal outcomes. Evidence from the recent animal experiments and pilot studies in babies suggest that other agents combined with hypothermia may enhance overall neuroprotection. This review discusses such neuroprotective agents.

Keywords: Neonatal encephalopathy; Neuroprotection; Hypothermia

Introduction

Neonatal encephalopathy (NE), following intrapartum hypoxia-ischemia, continues to be a significant cause of death and developmental disability in children. Every year, there are reported 4 million neonatal deaths worldwide and NE is attributed to approximately 23% of them [1]. In high-income countries such as UK, NE affects approximately 2 per 1000 neonates [2]. However, in low and middle-income countries such as India, it occurs in approximately 7-20 per 1000 neonates [3]. The spectrum of abnormal neurological outcome following NE could vary from cerebral palsy to significant disabilities including blindness, deafness and cognition problems [4,5].

Therapeutic hypothermia for infants with hypoxic ischemic encephalopathy has been extensively studied in preclinical models [6] and several major randomized trials [7-9]. Evidence from the trials suggest that moderate hypothermia for infants with hypoxic ischemic encephalopathy reduces death and disability [10]. Therapeutic hypothermia is now a standard practice in several countries [11]. Despite the remarkable benefits of hypothermia, one meta-analysis indicates that the composite adverse outcome following hypoxic ischemic encephalopathy reduces from 58% to 47% with hypothermia [12]. However therapeutic hypothermia lengthens the latent phase before the onset of secondary energy failure thereby expands the duration of the therapeutic window [13]. Neuroprotective agents, given during this time, may add incrementally to the proven benefit of hypothermia [14-16]. This review considers those agents and their neuroprotective ability to improve adverse outcome following hypoxic ischemic encephalopathy.

History & Background

Scientific studies of neonatal therapeutic hypothermia started in the 1950s when researchers like Bjorn Westin described their work in terms of re-animation rather than neuroprotection [17, 18]. Several other investigators carried out clinical observations and careful physiological experiments [18-22]. In the 1960s, however, clinicians saw hypothermia in newborn babies was something to be avoided.

The problem of infants who did not breathe at birth had been solved by the invention of mechanical ventilation, so any benefit of cooling was no longer needed, and a significant study showed that keeping small infants warm increased their survival [23]. These results, together with observational [24] and experimental [25] data made it an article of medical faith for years that babies should not be allowed to get cold. Although, in the Soviet Union, cooling was being applied empirically following birth asphyxia [26], the language barrier, cold war politics and the Russians' failure to carry out randomized controlled trials contributed to an almost total ignorance of this work in the West.

In the late 1980s, the development of a new set of concepts and problems led to a re-examination. The growing evidence that protecting the brain against the effects of oxygen deprivation during labour might be possible influenced a new generation of neonatal researchers. These researchers were aware that cooling produced powerful intra-ischaemic neuroprotection during cardiac surgery but a new concept of hypothermic post-insult neural rescue developed. This shift in thinking was possible because of few major new ideas that were developing at the same time: delayed post-ischemic cell death, excitotoxicity and apoptosis.

The first paradigm shift that influenced researchers in particular was the idea that if a baby was resuscitated after hypoxia-ischemia there was a period of time before brain cells started to die. Researchers at University College London used the newly developed technique of Magnetic Resonance Spectroscopy (MRS) to show that the infant brain metabolism is normal in the hours after birth asphyxia and deteriorated only after a distinct delay [27]. Robert Vannucci confirmed the effect [28] and delayed injury was also reported in neuropathological studies [29,30]. Delayed brain injury (called 'secondary energy failure') was a critical new idea. If brain cells remained normal for a time and the mechanism of the delayed death could be unraveled, it opened the possibility of therapeutic intervention [31].

The concept of excitotoxicity was due to the work of John Olney [32,33] and Brian Meldrum [34]. They showed that the neural cell death caused by hypoxia-ischemia is mediated by excess production of the excitatory neurotransmitter glutamate, and that pharmacological blockade of the N-methyl-D-aspartate receptor could provide good protection against hypoxic damage. The discovery of programmed cell death, a novel form of cell suicide was observed as a pathological appearance and named apoptosis ("falling off", as of leaves) in the 1970s [35], Horvitz [36], Raff [37], and Evan [38] provided a molecular understanding and showed that apoptosis could be triggered by cellular insults.

Neuroprotective agents

Xenon (Xe)

Xenon is a rare noble gas and is approved for use as a general anesthetic. Xenon is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor [39]. Other actions of Xenon are inhibition of calcium/calmodulin dependent protein kinase II [40], activation of anti-apoptotic effectors such as Bcl-XL and Bcl-2 [41], and activation of background two-pore K⁺ channel [42]. Xenon also activates the induced expression of hypoxia inducible factor 1 α and its downstream effectors erythropoietin and vascular endothelial growth factor, which can interrupt the apoptotic pathway [43].

In the animal studies, it has been shown that Xenon is neuroprotective following hypoxia - ischemia and synergistic effect was observed when administered with mild hypothermia [44,45]. This effect was retained even if xenon is delayed or asynchronously applied with hypothermia [46,47]. Xenon also has a remarkable safety profile, given its apparent hemodynamic stability and myocardial protective properties [48-50]. Xenon is a very expensive agent and must be scavenged and reused. Recently, a randomized controlled pilot study (Toby Xe) in UK has shown that 30% Xenon, when given (within 12 hours of birth) to newborn babies with moderate to severe encephalopathy along with hypothermia, did not enhance the neuroprotective effect of cooling [51]. Another phase 2 randomized control study (cool xenon 3 study) is currently underway in UK (clinical trials NCT02071394), aiming to give 50% Xenon along with hypothermia. In brief, the exact concentration and duration of xenon therapy needed for neuroprotection in newborn infants is yet to be established.

Melatonin

Melatonin is a naturally occurring hormone produced in the pineal gland, retina and gastrointestinal tract. Melatonin acts as an anti-

oxidant, scavenging hydroxyl and nitrosyl free radicals. It also stimulates other antioxidant enzymes including glutathione peroxidase and superoxide dismutase. Melatonin readily crosses blood brain barrier because of its lipophilic properties, and binds to specific receptors in brain.

Melatonin has been shown to be neuroprotective following brain injury in animal models. Neuroprotection was observed when melatonin was given before or after hypoxic-ischemic insult in a rat model with dose range between 5 and 15mg/kg [52,53]. Melatonin is beneficial in reducing neurotoxic effects on developing white matter in a mouse model [54]. Delayed administration of melatonin is also protective following transient focal hypoxic-ischemic insult in rats [55]. Melatonin also attenuates gray and white matter damage in a mouse model of transient focal hypoxic-ischemic insult [56]. Other neuroprotective effects of melatonin include anti-inflammatory [55] and anti-apoptotic activities [57]. Melatonin showed neuroprotection in fetal sheep when it was given after umbilical cord occlusion [58]. The optimal neuroprotective dose has yet to be determined, as wide range of melatonin doses were used in various species from 0.005mg/kg in newborn mice [54] to 20 mg/kg/hour in fetal sheep [58]. In newborn piglet model of asphyxia, a 5-mg/kg infusion for 6 hours started 10 minutes after resuscitation and repeated at 24 hours augmented hypothermic neuroprotection [59]. No adverse effects of melatonin were observed when it was evaluated in two small studies involving term newborn infants [60,61]. Recently, one clinical study involving small group of newborn infants with HIE, early administration of melatonin (10 mg/kg, enteral administration) had improved survival without neurological abnormalities at 6 months of age [62]. In summary, melatonin possesses multifaceted neuroprotective capacity and shows almost no toxicity in humans, it is likely that more clinical studies will commence in near future.

N-acetyl cysteine (NAC)

N-acetyl cysteine (NAC) is an amino acid supplement, and a precursor of glutathione. It has multiple actions including anti-oxidant, anti-inflammatory and anti-apoptotic activities. It has low toxicity, crosses the placenta and blood brain barrier. NAC has been widely used in children as an antidote for paracetamol intoxication and also for mucolysis. NAC reduces reperfusion injury and decreases inflammation and nitric oxide production in adult models of stroke [63]. NAC was observed to provide remarkable neuroprotection in animal model following lipopolysaccharide (LPS)-sensitized hypoxic-ischemic brain injury [64]. NAC also attenuates LPS induced inflammatory response and metabolism of phospholipids at fetomaternal interface in pregnant rats [65].

NAC reduced cerebral oxidative stress and cerebral lactate, improved cerebral perfusion in a piglet model of hypoxia-reoxygenation [66]. NAC combined with hypothermia, decreased infarct volume, improved myelin expression and functional outcomes in a rat model after focal hypoxic ischemic injury [16]. The neuroprotective effect of combined hypothermia and NAC is also shown in spinal cord ischemia [67].

In human studies, NAC was shown to achieve predictable plasma concentrations in fetus, when administered to pregnant women, indicating that antenatal neuroprotection may be possible for vulnerable babies [68]. NAC has already been studied in newborn babies, making it a safe drug to study as a neuroprotectant [69]. Currently, a study, using NAC and TH in newborn babies with HIE, is being done in Imperial College London (MARINAC study). We will know more about the value of NAC in neonatal neuroprotection in the near future.

Allopurinol

Allopurinol is a xanthine-oxidase inhibitor, used to treat chronic gout. Xanthine oxidase is an enzyme involved in superoxide production. In high concentrations, it also scavenges free radicals and prevents free radical formation by chelating with free iron. Thus, in high doses, it was found to be neuroprotective. In rat model, Allopurinol reduced cerebral edema and long term brain damage when given after cerebral hypoxia-ischemia [70]. When Allopurinol was given as pretreatment, it preserves cerebral energetics during perinatal hypoxia-ischemia [71].

Allopurinol reduced reperfusion injury in adults undergoing coronary bypass surgery [72]. When infants with hypoplastic left heart syndrome undergo deep hypothermic cardiac arrest, pre-treatment with Allopurinol reduces adverse postoperative neurological and

cardiac outcomes [73]. Recent Cochrane review suggested that larger trials are needed to assess the neuroprotective effect of allopurinol in conjunction with hypothermia in infants with moderate to severe encephalopathy as the meta-analysis did not reveal any statistical difference [74]. Allopurinol crosses the placenta and must be administered early for neuroprotection as it acts through free radical scavenging property. Maternal allopurinol administration during fetal hypoxia reduces cord blood levels of S-100B [75]. ALLO trial, a multicenter randomized controlled trial in the Netherlands, has recently shown that when allopurinol was given to pregnant women at term in whom the fetal hypoxia is suspected, it did not significantly lower neuronal damage markers in cord blood [76].

Erythropoietin (EPO)

Erythropoietin is a naturally occurring glycoprotein and a pleiotropic cytokine, which stimulates erythropoiesis in human neonates. Erythropoietin is a large molecule that crosses the blood brain barrier by active transport mechanism [77]. Epo receptors are situated throughout the central nervous system on neurons, glia and endothelial cells; they participate in proliferation and differentiation of these cells and is up-regulated in umbilical cord blood from asphyxiated babies [78]. Intracerebral production of Epo has been reported after hypoxic stress in neonates [79].

Epo appears to have multiple neuroprotective properties such as direct neurotropic effect, decreased susceptibility to glutamate toxicity, induction of anti-apoptotic factors, anti-inflammatory effects and anti-oxidant effects. The vital role for Epo is repair; Epo binding stimulates neurogenesis, oligodendrogenesis and angiogenesis. Evidence from preclinical and clinical studies showed the conditioning and regenerative potential of Epo, which is now emerging as a promising neuro protectant. Preclinical studies have suggested that Epo is neuroprotective following hypoxia-ischemia and reperfusion when given either before or after the hypoxic-ischemic insult [80,81]. Several clinical studies have shown Epo is safe in newborn babies and the neuroprotective effects of Epo. Early administration and high dose of Epo enhances long term neuroprotection and promote neurogenesis in neonatal infants following neonatal stroke [82]. A randomized control trial of term infants with moderate or severe hypoxic ischemic encephalopathy reported that adverse neurological outcome was significantly less in Epo treated infants (300 or 500 units/kg) [83]. A study of 45 term infants comparing single-dose Epo alone on day 0 with 72h therapeutic hypothermia alone for treatment of NE found superior protection in the hypothermia group [84]. A number of large studies are currently underway [85]. Complications seen in adults such as hypertension, clotting, seizures, polycythemia have not been observed in infants.

Anticonvulsants

Evidence from both animal and human studies has shown that seizures enhance neonatal hypoxic-ischemic damage [86,87], thus effective prevention of seizures could attenuate brain injury. Two anticonvulsants of particular interest are topiramate and levetiracetam.

Topiramate is widely used as an anticonvulsant in paediatric population [88] and it has several mechanisms of action. It modulates inhibitory gamma-aminobutyric acid(GABA)-mediated receptors and blocks presynaptic alpha-amino-3 hydroxy-5 methyl-4isoxazolepropionate(AMPA) excitatory channels and modulates voltage-activated Na⁺ and Ca⁺⁺ channels, which may reduce excessive release of glutamate following hypoxia-ischemia [89-91].

Topiramate, when combined with hypothermia, improved functional performance and reduced the severity of brain injury in a rat stroke model [15]. Topiramate also suppresses acute seizures and reduced neuronal fragmentation in a rat pup model of asphyxia [92]. In a p7 rodent model, topiramate was found to be neuroprotective by reducing white matter injury following hypoxic-ischemic insult and decreased motor deficits [93]. In a piglet model of hypoxia-ischemia, topiramate, when given in high doses (20 mg/kg) compared to low doses (10 mg/kg) markedly reduced the severity of neuronal injury [94]. However, further studies are needed to assess the effect of hypothermia on the metabolism of topiramate as it has been shown that therapeutic hypothermia reduces both absorption and elimination of topiramate [95].

Levetiracetam is the S-enantiomer of etiracetam. The US FDA approved it in 1999 and by UK for epilepsy recently. It has become one of the first line anticonvulsant recently. Neuroprotective properties of Levetiracetam were shown in animal model of focal cerebral isch-

emia [96]. In a recent pilot study in neonatal infants, there were no adverse effects noted when Levetiracetam was used to treat neonatal seizures [97] and also it was noted that it does not induce cell death in the developing brain even at high dose [98].

Conclusion and Future directions in research

Over the past couple of decades, significant advancement has been made in neuroprotection for neonates. Many years of clinical and basic science research into the pathogenesis of HIE in term infants have been translated into the clinical use of therapeutic hypothermia for infants with HIE, and it is standard of care for neonates with HIE in many countries currently. There is also great potential to augment hypothermic neuroprotection with co-administered neuroprotective agents.

Further preclinical and clinical studies are needed to demonstrate the best neuroprotective agent when combined with hypothermia to provide effective neuroprotection following hypoxic ischemic encephalopathy. It is vital to ensure that developing brain is not further affected by the use of newer drugs when tried alone or in combination with others. The challenge will be to ensure the timing and dose of each neuroprotectant, which are appropriate for the phase of injury to ensure optimal and lasting protection. Eventually, it seems that in the future, a cocktail of multiple neuroprotective agents will be used to treat neonates with HIE that will act on different pathways in the pathogenesis. Moreover, prediction and prevention of at risk fetus before delivery is essential. Hence, clinical tools to reliably predict term fetuses at risk for HIE and strategies to ameliorate that risk before brain injury occurs are vital.

Bibliography

1. Lawn J E., *et al.* "4 million neonatal deaths: when? Where? Why?" *Lancet* 365.9462 (2005): 891-900.
2. Pierrat V., *et al.* "Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy: population based study". *Archives of Disease in Childhood - Fetal and Neonatal Edition Journal* 90.3 (2005): 257-261.
3. Lawn J E., *et al.* "Two million intrapartum-related stillbirths and neonatal deaths: where, why, and what can be done?" *International Journal of Gynecology & Obstetrics* 107 (2009): S5-S18.
4. Al-Macki N., *et al.* "The spectrum of abnormal neurologic outcomes subsequent to term intrapartum asphyxia". *Pediatric Neurology* 41.6 (2009): 399-405.
5. Rennie J M., *et al.* "Outcome after intrapartum hypoxic ischaemia at term". *Seminars in Fetal and Neonatal Medicine* 12.5 (2007): 398-407.
6. Bona E., *et al.* "Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: short- and long-term outcome". *Pediatric Research* 43.6 (1998): 738-745.
7. Azzopardi D V., *et al.* "Moderate hypothermia to treat perinatal asphyxial encephalopathy". *New England Journal of Medicine* 361.14 (2009): 1349-1358.
8. Shankaran S., *et al.* "Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy". *New England Journal of Medicine* 353.15 (2005): 1574-1584.
9. Jacobs SE., *et al.* "Whole-body hypothermia for term and near-term newborns with hypoxic-ischemic encephalopathy: a randomized controlled trial". *Archives of Pediatrics and Adolescent Medicine* 16.5 (2011) 692-700.
10. Tagin M A., *et al.* "Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis". *Archives of Pediatrics and Adolescent Medicine* 166.6 (2012): 558-566.
11. Kapetanakis A., *et al.* "Therapeutic hypothermia for neonatal encephalopathy: a UK survey of opinion, practice and neuro-investigation at the end of 2007". *Acta Paediatrica* 98.4 (2009): 631-635.

12. Edwards A D, *et al.* "Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data". *BMJ* 2010: 340-363.
13. O'Brien F E, *et al.* "Delayed whole-body cooling to 33 or 35 degrees C and the development of impaired energy generation consequential to transient cerebral hypoxia-ischemia in the newborn piglet". *Pediatrics* 117.5 (2006): 1549-1559.
14. Ma D, *et al.* "Xenon and hypothermia combine to provide neuroprotection from neonatal asphyxia". *Annals of Neurology* 58.2 (2005): 182-193.
15. Liu Y, *et al.* "Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats". *Stroke* 35.6 (2004): 1460-1465.
16. Jatana M, *et al.* "Combination of systemic hypothermia and N-acetylcysteine attenuates hypoxic-ischemic brain injury in neonatal rats". *Pediatric Research* 59.5 (2006): 684-689.
17. Wang H, *et al.* "Cold as a therapeutic agent". *Acta Neurochir (Wien)* 148.5 (2006): 565-570.
18. Westin B, *et al.* "Hypothermia in the resuscitation of the neonate: a glance in my rear-view mirror". *Acta Paediatrica* 95.10 (2006): 1172-1174.
19. Miller J A, *et al.* "Factors in Neonatal Resistance to Anoxia. I. Temperature and Survival of Newborn Guinea Pigs Under Anoxia." *Science* 110.2848 (1949): 113-114.
20. Enhorning G and B Westin. "Experimental studies of the human fetus in prolonged asphyxia". *Acta Physiologica Scandinavica* 31.4 (1954): 359-375.
21. Westin B, *et al.*, "Neonatal asphyxia pallida treated with hypothermia alone or with hypothermia and transfusion of oxygenated blood". *Surgery* 45.5 (1959): 868-879.
22. Auld PA, *et al.* "Physiologic studies on an infant in deep hypothermia". *New England Journal of Medicine* 1962. 267: 1348-51.
23. Silverman W A, *et al.* "The influence of the thermal environment upon the survival of newly born premature infants". *Pediatrics* 22.5 (1958): 876-886.
24. Mann T P, *et al.* "Neonatal cold injury". *Med World* 90.4 (1959): 321-326.
25. Brodie H R, *et al.* "Heat production in new-born infants under normal and hypoxic conditions". *The Journal of Physiology* 138.1 (1957): 156-163.
26. Kopshev S N, *et al.* "[Cranio-cerebral hypothermia in the prevention and combined therapy of cerebral pathology in infants with asphyxia neonatorum]". *Akush Ginekol (Mosk)* 7 (1982): 56-58.
27. Delpy, D T, *et al.* "Noninvasive investigation of cerebral ischemia by phosphorus nuclear magnetic resonance". *Pediatrics* 70.2 (1982): 310-313.
28. Vannucci R C, *et al.* "Experimental biology of cerebral hypoxia-ischemia: relation to perinatal brain damage". *Pediatric Research* 27.4 (1990): 317-326.
29. Kirino T, *et al.* "Delayed neuronal death in the gerbil hippocampus following ischemia". *Brain Research* 239.1 (1982): 57-69.
30. Pulsinelli W A, *et al.* "Temporal profile of neuronal damage in a model of transient forebrain ischemia". *Annals of Neurology* 11.5 (1982): 491-498.

31. Hope PL, *et al.* "Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants". *Lancet* 2.8399 (1984): 366-370.
32. Olney J W and LG Sharpe "Brain lesions in an infant rhesus monkey treated with monosodium glutamate". *Science* 166.3903 (1969): 386-388.
33. Olney J W and O L Ho "Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine". *Nature* 227.5258 (1970): 609-611.
34. Simon R P, *et al.* "Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain". *Science* 226.4676 (1984):850-852.
35. Kerr J F, *et al.* "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics". *British Journal of Cancer* 26.4 (1972): 239-257.
36. Horvitz H R, *et al.* "Genetic control of programmed cell death in the nematode *Caenorhabditis elegans*". *Cancer Research* 59 (1999): 1701s-1706s.
37. Raff M C, *et al.* "Social controls on cell survival and cell death". *Nature* 356.6368 (1992): 397-400.
38. Evan G I, *et al.* "Induction of apoptosis in fibroblasts by c-myc protein". *Cell* 69.1 (1992): 119-128.
39. Franks N P, *et al.* "How does xenon produce anaesthesia?" *Nature* 396.6709 (1998): 324.
40. Petzelt C P, *et al.* "Participation of the Ca (2+)-calmodulin-activated Kinase II in the control of metaphase-anaphase transition in human cells". *Cell Biology International* 25.5 (2001): 403-409.
41. Ma D, *et al.* "Xenon mitigates isoflurane-induced neuronal apoptosis in the developing rodent brain". *Anesthesiology* 106. 4 (2007): 746-753.
42. Gruss M, *et al.* "Two-pore-domain K⁺ channels are a novel target for the anesthetic gases xenon, nitrous oxide, and cyclopropane". *Molecular Pharmacology* 65.2 (2004): 443-452.
43. Ma D, *et al.* "Xenon preconditioning protects against renal ischemic-reperfusion injury via HIF-1 α activation". *Journal of the American Society of Nephrology* 20.4 (2009): 713-720.
44. Chakkarapani E, *et al.* "Xenon enhances hypothermic neuroprotection in asphyxiated newborn pigs". *Annals of Neurology* 68.3 (2010): 330-341.
45. Faulkner S, *et al.* "Xenon augmented hypothermia reduces early lactate/N-acetylaspartate and cell death in perinatal asphyxia". *Annals of Neurology* 70.1 (2011): 133-150.
46. Thoresen M, *et al.* "Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia". *Journal of Cerebral Blood Flow & Metabolism* 29.4 (2009): 707-714.
47. Martin J L, *et al.* "Asynchronous administration of xenon and hypothermia significantly reduces brain infarction in the neonatal rat". *British Journal of Anaesthesia* 98.2 (2007): 236-240.
48. Coburn M, *et al.* "Randomized controlled trial of the haemodynamic and recovery effects of xenon or propofol anaesthesia". *British Journal of Anaesthesia* 94.2 (2005): 198-202.
49. Preckel B, *et al.* "Xenon produces minimal haemodynamic effects in rabbits with chronically compromised left ventricular function". *British Journal of Anaesthesia* 88.2 (2002): 264-269.

50. Preckel B., *et al.* "The direct myocardial effects of xenon in the dog heart *in vivo*". *Anaesthesia Analog* 94.3 (2002): 545-551.
51. Azzopardi D., *et al.* "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* (2015).
52. Pei, Z., *et al.* "Pretreatment with melatonin reduces volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model". *Journal of Pineal Research* 32.3 (2002): 168-172.
53. Pei, Z., *et al.* "Administration of melatonin after onset of ischemia reduces the volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model". *Stroke* 34.3 (2003): 770-775.
54. Husson I., *et al.* "Melatonergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge". *Annals of Neurology* 51.1 (2002): 82-92.
55. Lee M Y., *et al.* "Intravenous administration of melatonin reduces the intracerebral cellular inflammatory response following transient focal cerebral ischemia in rats". *Journal of Pineal Research* 42.3 (2007): 297-309.
56. Lee E J., *et al.* "Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia". *Journal of Pineal Research* 38.1 (2005): 42-52.
57. Chen H Y., *et al.* "Melatonin decreases neurovascular oxidative/nitrosative damage and protects against early increases in the blood-brain barrier permeability after transient focal cerebral ischemia in mice." *Journal of Pineal Research* 41.2 (2006): 175-182.
58. Welin A K., *et al.* "Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion". *Pediatric Research* 61.2 (2007): 153-158.
59. Robertson N J., *et al.* "Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model". *Brain* 136 (2013): 90-105.
60. Gitto E., *et al.* "Effects of melatonin treatment in septic newborns". *Pediatric Research* 50.6 (2001): 756-60.
61. Fulia F., *et al.* "Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin". *Journal of Pineal Research* 31.4 (2001): 343-349.
62. Aly H., *et al.* "Melatonin use for neuroprotection in perinatal asphyxia: a randomized controlled pilot study". *Journal of Perinatology* 35.3 (2015): 186-191.
63. Khan M., *et al.* "Administration of N-acetylcysteine after focal cerebral ischemia protects brain and reduces inflammation in a rat model of experimental stroke". *Journal of Neuroscience Research* 76.4 (2004): 519-527.
64. Wang X., *et al.* "N-acetylcysteine reduces lipopolysaccharide-sensitized hypoxic-ischemic brain injury". *Annals of Neurology* 61.3 (2007): 263-271.
65. Paintlia M K., *et al.* "Attenuation of lipopolysaccharide-induced inflammatory response and phospholipids metabolism at the fetomaternal interface by N-acetyl cysteine". *Pediatric Research* 64.4 (2008): 334-339.
66. Lee T F., *et al.* "Effects of postresuscitation N-acetylcysteine on cerebral free radical production and perfusion during reoxygenation of hypoxic newborn piglets". *Pediatric Research* 64.3 (2008): 256-261.
67. Cakir O., *et al.* "Neuroprotective effect of N-acetylcysteine and hypothermia on the spinal cord ischemia-reperfusion injury". *Cardiovascular Surgery* 11.5 (2003): 375-379.
68. Wiest D B., *et al.* "Antenatal pharmacokinetics and placental transfer of N-acetylcysteine in chorioamnionitis for fetal neuroprotection". *Journal of Pediatrics* 165.4 (2014): 672-677.

69. Ahola T, *et al.* "N-acetylcysteine does not prevent bronchopulmonary dysplasia in immature infants: a randomized controlled trial". *Journal of Pediatrics* 143.6 (2003): 713-719.
70. Palmer C., *et al.* "Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats". *Pediatrics Research* 33 (1993): 405-411.
71. Williams G D., *et al.* "Allopurinol preserves cerebral energy metabolism during perinatal hypoxia-ischemia: a ³¹P NMR study in un-anesthetized immature rats". *Neuroscience Letters* 144 (1992): 103-106.
72. Johnson W D., *et al.* "A randomized controlled trial of allopurinol in coronary bypass surgery". *American Heart Journal* 121 (1991): 20-24.
73. Clancy R R., *et al.* "Allopurinol neurocardiac protection trial in infants undergoing heart surgery using deep hypothermic circulatory arrest". *Pediatrics* 108.1 (2001): 61-70.
74. Chaudhari T and W McGuire. "Allopurinol for preventing mortality and morbidity in newborn infants with hypoxic-ischaemic encephalopathy". *Cochrane Database of Systematic Reviews* (2012).
75. Torrance H L., *et al.* "Maternal allopurinol during fetal hypoxia lowers cord blood levels of the brain injury marker S-100B". *Pediatrics* 124.1 (2009): 350-357.
76. Kaandorp J J., *et al.* "Maternal allopurinol administration during suspected fetal hypoxia: a novel neuroprotective intervention? A multicentre randomised placebo controlled trial". *Arch Dis Child Fetal Neonatal Ed* 100.3 (2015): F216-F223.
77. Brines M L., *et al.* "Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury". *Proceedings of the National Academy of Sciences U S A* 97.19 (2000): 10526-10531.
78. Ruth V., *et al.* "Postnatal changes in serum immunoreactive erythropoietin in relation to hypoxia before and after birth". *Journal of Pediatrics* 116.6 (1990): 950-954.
79. Juul S E., *et al.* "Erythropoietin in the cerebrospinal fluid of neonates who sustained CNS injury". *Pediatrics Research* 46.5 (1999): 543-547.
80. van der Kooij M A., *et al.* "Neuroprotective properties and mechanisms of erythropoietin in in vitro and in vivo experimental models for hypoxia/ischemia". *Brain Research Reviews* 59.1 (2008): 22-33.
81. Maiese K., *et al.* "New avenues of exploration for erythropoietin". *JAMA* 293.1 (2005): 90-95.
82. Gonzalez F F., *et al.* "Erythropoietin enhances long-term neuroprotection and neurogenesis in neonatal stroke". *Developmental Neuroscience* 29. (4-5) (2007): 321-330.
83. Zhu C., *et al.* "Erythropoietin improved neurologic outcomes in newborns with hypoxic-ischemic encephalopathy". *Pediatrics* 124.2 (2009): e218-e226.
84. El Shimi M S., *et al.* "Single dose recombinant erythropoietin versus moderate hypothermia for neonatal hypoxic ischemic encephalopathy in low resource settings". *Journal of Maternal-Fetal and Neonatal Medicine Impact* 27.3 (2014): 1295-300.
85. Rangarajan V and S E Juul. "Erythropoietin: emerging role of erythropoietin in neonatal neuroprotection". *Pediatric Neurology* 51.4 (2014): 481-488.
86. Glass H C., *et al.* "Clinical Neonatal Seizures Are Independently Associated with Outcome in Infants at Risk for Hypoxic-Ischemic Brain Injury". *Journal of Pediatrics* 155.3 (2009): 318-323.

87. Wirrell E C., *et al.* "Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage". *Pediatric Research* 50.4 (2001): 445-454.
88. Kim J M., *et al.* "Long-term effectiveness and tolerability of topiramate in children with epilepsy under the age of 2 years: 4-year follow-up". *Journal of Korean Medical Science* 24.6 (2009): 1078-1082.
89. Taverna S., *et al.* "Inhibition of transient and persistent Na⁺ current fractions by the new anticonvulsant topiramate". *Journal of Pharmacology and Experimental Therapeutics* 288.3 (1999): 960-968.
90. Zona C M T Ciotti and M Avoli. "Topiramate attenuates voltage-gated sodium currents in rat cerebellar granule cells". *Neuroscience Letters* 231.3 (1997): 123-126.
91. Herrero A I., *et al.* "Two new actions of topiramate: inhibition of depolarizing GABA(A)-mediated responses and activation of a potassium conductance". *Neuropharmacology* 42.2 (2002): 210-220.
92. Koh S and F E Jensen. "Topiramate blocks perinatal hypoxia-induced seizures in rat pups". *Annals of Neurology* 50.3 (2001): 366-372.
93. Follett P L., *et al.* "Glutamate receptor-mediated oligodendrocyte toxicity in periventricular leukomalacia: a protective role for topiramate". *Journal of Neuroscience* 24.18 (2004): 4412-4420.
94. Schubert S., *et al.* "Neuroprotective effects of topiramate after hypoxia-ischemia in newborn piglets". *Brain Research* 1058. (1-2) (2005): 129-136.
95. Filippi L., *et al.* "Topiramate concentrations in neonates treated with prolonged whole body hypothermia for hypoxic ischemic encephalopathy". *Epilepsia* 50.11 (2009): 2355-2361.
96. Hanon E and H Klitgaard. "Neuroprotective properties of the novel antiepileptic drug levetiracetam in the rat middle cerebral artery occlusion model of focal cerebral ischemia". *Seizure* 10.4 (2001): 287-93.
97. Furwentsches A., *et al.* "Levetiracetam in the treatment of neonatal seizures: a pilot study". *Seizure* 19.3 (2010): 185-199.
98. Kim J S., *et al.* "Neurodevelopmental impact of antiepileptic drugs and seizures in the immature brain". *Epilepsia* 48. (2007): 19-26.

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