We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 5

Perinatal and Neonatal Hypoxia Ischaemia: The Unique Challenges of Treating the Infant Brain

Lancelot Jamie Millar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79674

Abstract

Hypoxic ischaemic injury can damage the brain at any age. However, the infant brain displays a unique profile of sensitivity and resistance compared to adult ischaemic stroke patients. Both pathology and response to treatment are uniquely affected by the molecular landscape of the neonatal brain. With new revelations in the biology of brain injury in perinates and neonates being discovered, as global mortality and morbidity increases research funding into infant brain injury, it is important to raise awareness of the unparalleled challenge of treating these young patients. This chapter will review currently known differences between the infant and adult brain response to hypoxia, and address existing treatments alongside proposed treatments not yet evaluated by clinical trial.

Keywords: perinatal, neonatal, hypoxia, ischaemia, ischemia, hypothermia, development

1. Introduction

The clinical definition of neonatal hypoxic ischaemic (HI) injury is "asphyxia of the umbilical blood supply to the human foetus occurring at 36 gestational weeks or later" [1–4]. Neonatal HI has also been referred to as hypoxic-ischaemic encephalopathy (HIE), where the neonatal period is interchangeably referred to as "term" [2, 4]. If the injury occurs prior to 36 gestational weeks, the condition is described as perinatal hypoxia ischaemia.

Neonatal hypoxia ischaemia is diagnosed based on a range of factors which correlate with clinical outcome [1, 5, 6]. These include: 5-min Apgar score of less than 5 [7, 9]; need for delivery room intubation or cardiopulmonary resuscitations [8]; umbilical cord arterial pH below 7.00 [9]; and absence of normal neurological signs, such as the infant sucking reflex

[7, 10]. These are only a selection of risk factors assessed postnatally, and there is an enormous range of clinical outcomes amongst infant patients diagnosed with HI [11, 12].

Globally, hypoxia ischaemia is the single most common cause of death and disability in human neonates [13–15], making further research into pathophysiology and treatment an international priority. Persistent disability is common is surviving infants. Clinical outcomes can range from death to normal neurological profile at 2 years follow-up [16]. Meta-analysis studies have documented that 5–10% of patients developed a persistent motor disability, with up to 50% of patients displaying cognitive or sensory disorders in childhood or adolescence [17–20]. Between 0.7 and 1.2 million infants are born with evidence of hypoxic ischaemic brain injury every year, accounting for 23% of global infant mortality [21]. Survival rates have increased since the 1990s [22], perhaps in part due to improvements in intensive care technology, yet the prevalence of morbidity associated with infant HI remains undiminished [23, 24]. These sobering statistics should draw greater attention to the study of hypoxia in the developing brain, and the need for protective therapies to administer in these vulnerable infants.

2. The unique molecular landscape of the infant brain

Hypoxia exacts damage on the neonatal brain in a unique profile incomparable to the effect of ischaemic stroke on the adult brain. The fundamental anatomy and chemistry of the immature brain creates sites of increased sensitivity and resistance, many of which basic science is only beginning to understand [16, 25]. This chapter will examine several key areas where the physiology of the neonatal brain, and its susceptibility to hypoxic brain damage, requires special consideration. The section will cover: the effect of structural immaturity on the development of hypoxic ischaemic brain injury; alterations to the balance of cell death cascades; and the surprising sex differences in severity of neonatal injury.

2.1. Hypoxia ischaemia and the structurally immature brain

The basic anatomy of the foetal brain is far from the oxygen-rich vasculated tissue familiar from the adult brain, as summarised in **Figure 1**. Outlining the full range of age-dependent processes is beyond the scope of this chapter, but one excellent review [25] expands substantially on the information presented here.

The cerebral microvasculature is known to exhibit a significant risk of rupture, especially in premature neonates [26, 27]. Fluctuations in cerebral blood flow have been correlated with increased rates of intracerebral haemorrhage in infant patients [26, 28], an effect enhanced by altered CO_2 partial pressure in the blood [29] and haematocrit levels [30]. One influential model [31] of the neonatal blood brain barrier (BBB) describes the cerebral vasculature as undergoing a state of flux, remodelling vessels from basal-ganglia dense to a predominantly cortex enriched state. This immature, incomplete vascular structure has not formed permanent vessels by the time of birth. Research in animal models support this assessment, suggesting that neonatal blood vessels are surrounded by fewer astrocyte end-feet [32], demonstrating that the regulatory basement membrane which surrounds mature blood vessels is still forming in the neonatal brain.

http://dx.doi.org/10.5772/intechopen.79674

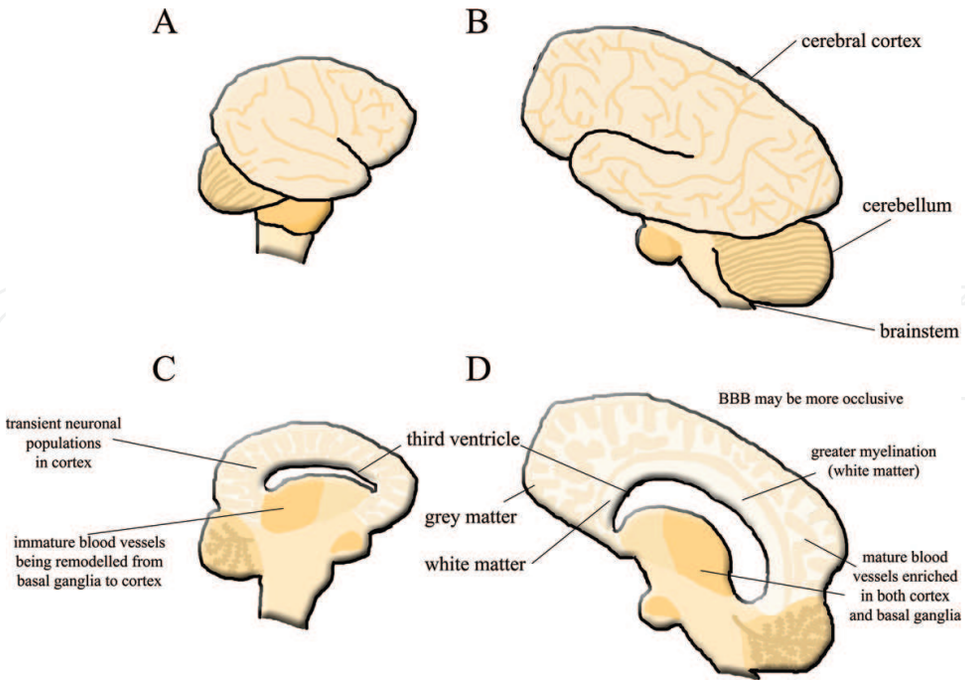

Figure 1. Schematic summarising gross anatomical differences between the adult and neonatal human brain. Side profile of whole neonatal (A) and adult (B) human brains. Sagittal cross-section of neonatal (C) and adult (D) human brains, revealing structure visible by magnetic resonance imaging (MRI). Several key differences are highlighted between the structure of the brain around birth and in the mature adult. BBB = blood brain barrier.

The infant cerebrovasculature is often described as operating on a "pressure passive" autoregulatory system [29]. Impaired vascular autoregulation has been reported as a risk factor for poor clinical outcome in cases of perinatal and neonatal hypoxia ischaemia, or other infant brain injuries [33, 34]. Low vascular tolerance of fluctuations in arterial CO_2 partial pressure and mean arterial blood pressure have been associated with severity of brain lesion in human patients [35]. Nitric oxide synthetase (NOS) inhibitors were shown to be effective at increasing tolerance of hypertension in neonatal pigs by increasing the upper cerebral blood flow limit for vascular autoregulation [36], an effect not replicated in juvenile animals. Currently, the mechanisms behind this "pressure passive "vascular regulation seen in the neonate remain unknown [25], yet the success of NOS in preserving the cerebral vasculature of neonatal pigs suggests that different molecules drive vascular autoregulation in the developing brain compared to the adult.

Another key differentiating factor between the adult and infant brain is the blood brain barrier. The BBB is composed of capillary endothelial cells, astrocytes, pericytes, and the basement membrane, forming a structure that regulates the transport of molecules between the blood and the extracellular matrix of the brain. The accepted view in the literature for some time has been that the immature BBB is less occlusive than that of the adult, enhancing brain damage when the infant brain is subjected to hypoxia ischaemia [16, 25].

Some researchers have reported increased 'leakage' through the BBB in the immature brain. In postnatal day 7 (P7) rat pups subjected to unilateral common carotid artery occlusion followed by exposure to hypoxia, BBB permeability to immunoglobulin G (IgG) was increased compared to P14 rats undergoing the same procedure [37]. When blood brain barrier transfer coefficient was measured in perinatal and neonatal sheep, a greater vulnerability to hyperosmolarity was

detected compared to postnatal sheep [38]. Conversely, matrix metalloproteinase 9 (MMP9) knock-out mice, which display reduced BBB permeability to IgG, were protected against neonatal HI, displaying reduced brain lesion size [39]. Pharmacophores which reduce BBB leakage are also protective [40, 41].

However, assumptions concerning the vulnerability of the BBB are now coming under revision [25]. Some experiments suggest that the increased BBB permeability in young rodents is a secondary consequence of brain inflammation [42, 43], which suggests that reducing inflammation in the hypoxic ischaemic brain may preserve BBB function. It is now known that tight junctions, the molecular structures within the BBB responsible for its occlusive properties, are present from the day embryonic blood vessels invade the foetal brain [16, 25, 44]. These foetal BBB units have been demonstrated to possess occlusive properties, excluding water molecules in the developing opossum brain [44, 45], and in piglets subjected to hypoxia ischaemia [46].

This brief overview highlights the importance of immature brain anatomy to the creating a unique set of factors influencing the outcome of hypoxic ischaemic brain injury in the infant brain. More basic research is needed to clarify the structure and functional capacities of the cerebral microvasculature in the perinatal and neonatal brain. This information will be essential prior to development of future therapies for oral or intravenous administration.

2.2. Cell death in the neonatal brain: excitotoxicity, oxidative stress, and inflammation

There are several other areas of divergence between the infant and adult brain in addition to vascular architecture. The immature brain also responds differently to major molecular cell death pathways. Hypoxia ischaemia mediates brain damage through three overlapping molecular cell death cascades: excitotoxicity, oxidative stress, and brain inflammation [16, 25], summarised in **Figure 2**. The following section will outline the unique vulnerability of the developing brain to each of these processes.

Excitotoxicity is a major cause of cell death in hypoxic ischaemic brain injury. During excitotoxicity, over-activation of physiological glutamate neurotransmission leads to excessive influx of positive ions through postsynaptic receptors, leading to cell death [16, 25, 47]. The *N*-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor stimulated during excitotoxicity, is expressed at a substantially higher level in the developing brain compared to the adult. In P6 rats, the NMDA receptor is expressed at 150–200% of adult levels [48]. The combination of NMDA receptor subunits also differs in the perinatal period. The subunits expressed in foetal rat favour prolonged calcium influx for a given excitation [49], increasing the sensitivity of the immature brain to excitotoxicity. Intracerebral NMDA injection in rats produces more extensive cell death in the neonate than in the adult [50]. Increased glutamate concentrations have also been documented in the cerebrospinal fluid (CSF) of human infants who have suffered severe HI injury [51].

Many factors contribute to the sensitivity of neurons to excitotoxic cell death, which is not solely mediated by NMDA receptors. Much of this unique molecular landscape remains to be understood. For example, it is known that neonatal brain is more prone to seizure activity than the adult brain [52], with severe seizures potentially resulting in permanent brain damage by

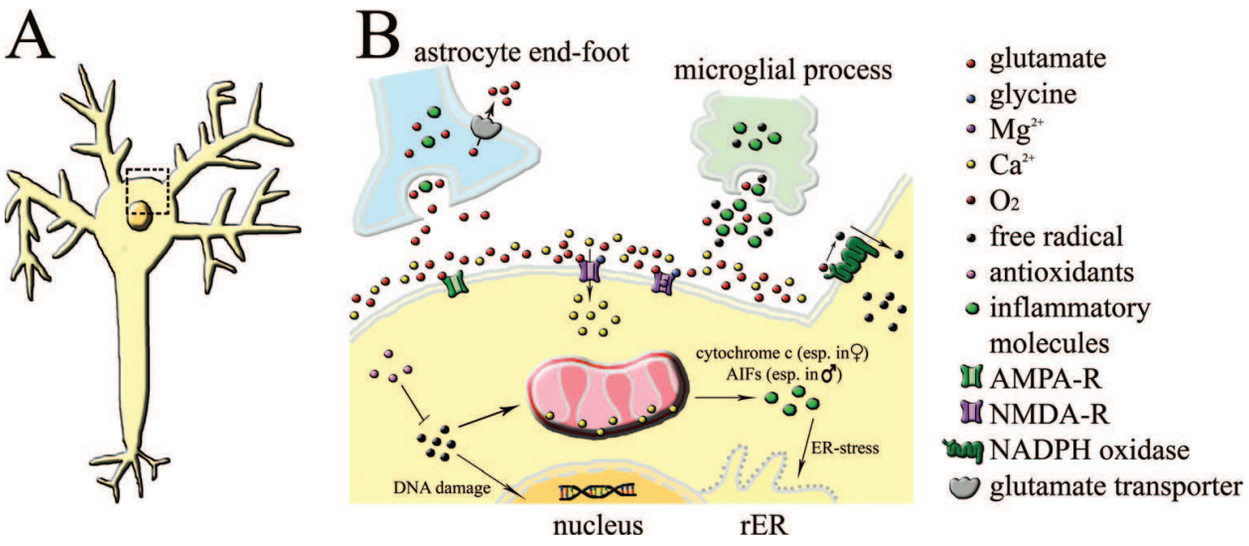

Figure 2. Schematic showing major molecular cascades contributing to the neuropathology of neonatal hypoxia ischaemia. (A) Schematic of an immature cortical neuron. (B) Sub-cellular molecular interactions in neonatal hypoxia ischaemia. The inset corresponds to the area of the neurons in panel A outlined by the dotted line. Molecules involved in excitotoxicity, oxidative stress, and inflammation closely interact. rER = rough endoplasmic reticulum, AIF = apoptosis-inducing factors, free radicals in H_2O_2, O_2^-, NO, inflammatory molecules include interleukins (IL1β, IL6), tumour necrosis factor alpha (TNFα), cytokines.

excitotoxic mechanisms. However, the mechanisms behind this sensitivity remain debated [52, 53]. Perinatal exposure to hypoxia is known to elicit seizures in rodent models [54]. Yet it is not clear if this is caused by the unique receptor complement of the developing brain, transcriptional responses to hyperexcitability, long-term remodelling responses to inflammation, or the paradoxical excitatory activity of the neurotransmitter γ-amino butyric acid (GABA) in the developing brain [53].

The oxidative stress molecular cell death cascade is integrally linked to that of excitotoxicity. Oxidative stress is the term for high levels of free radical production generated during oxygen metabolism under pathological conditions [55]. Hyperexcitability causes energy depletion, mitochondrial dysfunction, and calcium ion accumulation in the cytoplasm, which in turn lead to generation of free radicals, the damaging particles responsible for oxidative stress, which then trigger increased excitotoxicity [16, 55]. Free radicals are atoms or molecules containing an unpaired valence electron which makes these molecules highly chemically reactive and capable of stripping electrons from other molecules in the brain, particularly in the mitochondria [55].

In the adult brain, there exist several protective mechanisms which reduce the damage caused by oxidative stress, such as stores of antioxidants and nucleic acid or protein repair enzymes, which are not yet fully developed in the infant brain [56]. Expression of the enzyme nitric oxide synthetase (NOS), which inhibits mitochondrial respiration and generates free radicals based on NO, is up to 250% higher in the early postnatal rodent brain than in the adult [57]. Free radical scavenging cascades, which render these highly reactive molecules harmless, are present in the neonatal brain but less effective than in the adult brain [55, 58]. Immature oligodendrocytes were far less effective at degrading the free radical H_2O_2 *in vitro*, where scavenger

enzyme catalase was expressed at constant levels throughout development, but glutathione peroxidase was expressed at less than half of adult levels in oligodendrocytes from the neonatal brain [58]. There is also evidence that the scavenging cascades are less organised in the developing brain, with some rodent studies documenting decreased expression of key enzymes following exposure to hypoxia ischaemia [59].

In the first minutes after birth, the low oxygen environment of the foetus abruptly experiences an increase in O_2 partial pressure, which creates a pro-oxidant condition highly susceptible to oxidative stress prior to the development of healthy protective mechanisms [60]. Another potential reason for the vulnerability of the infant brain is the high polyunsaturated fat content, particularly in the white matter, making this region vulnerable to lipid peroxidation [61, 62]. Rodent studies have found that neonatal neurons may contain as little as a quarter of the full complement of mitochondria expressed in adult cells, with those neonatal mitochondria exhibiting altered calcium metabolism and internal matrix density as assessed by electron microscopy [63]. The complex molecular response to free radical generation is still only beginning to be understood in the infant brain.

The final factor known to contribute to neonatal hypoxic ischaemic brain injury is intracerebral inflammation [16, 64]. In humans, intrauterine infection is strongly associated with preterm birth and brain injury [64, 65]. In one long-term study, over 1000 premature infants diagnosed with early- or late-onset sepsis at birth were assessed for neurodevelopmental outcome at the age of five [66]. There was a strong correlation between sepsis at birth and diagnosis of cerebral palsy at age five, however, there was no correlation between sepsis and milder cognitive impairments. Although the infections were successfully treated in these patients, it is clear that there are persistent effects of infection-induced inflammation.

Molecular biology experiments in animal models have directly linked brain inflammation to neuronal cell death. Intracerebral inflammation triggered by injection of bacterial cell wall component lipopolysaccharide (LPS) caused neurodegeneration in young mice via activation of Toll-like receptor 4 [67]. One study investigating the effect of administering a single dose of LPS prior to hypoxia ischaemia in neonatal rats found lesion size increased by more than 100% compared to littermate animals that underwent hypoxia ischaemia alone [68]. This sensitivity to hypoxia in the presence of brain inflammation has been termed the "double-hit hypothesis" [69]. Interestingly, the injury-exacerbating effect of LPS on hypoxic ischaemic brain lesions may be specific to the infant brain. In one investigation, low-dose pre-treatment with intrauterine LPS increased injury severity in neonatal hypoxic ischaemic mouse, whereas the same pre-treatment was protective in adult animals [70, 71].

Despite the potential for inflammation to cause injury in animal models of infant hypoxia ischaemia, not all elements of the brain's inflammatory response are necessarily detrimental. The resident macrophages of the central nervous system, known as microglia, are activated within hours of the hypoxic ischaemic insult [72]. Microglia are known to produce a range of cytokines, excitotoxic neurotransmitter glutamate, and molecules known to induce oxidative stress such as nitric oxide and free radicals [16]. Additionally, chloroquine and minocycline, drugs which inhibit microglia and monocytes, decreased lesion size in a mouse model of neonatal hypoxia ischaemia [73]. However, microglia are complex secretory powerhouses with

http://dx.doi.org/10.5772/intechopen.79674

multiple active states, and there is growing support for a balanced understanding of these neuronal support cells as capable of causing both damaging and beneficial effects in neonatal hypoxia ischaemia [74, 75]. For example, when microglia were depleted in the brains of neonatal mice, lesion volume increased, along with the concentration of various cytokines and reactive oxygen species in the neonatal brain [76]. This suggests a neuroprotective function for microglia, at least under specific conditions.

2.3. Sexual dimorphism in the response to neonatal hypoxia ischaemia

One finding clearly illustrates how much remains to be understood about the neuropathology of neonatal hypoxia ischaemia. This is the recent discovery of sexual dimorphism in the developmental outcome of HI in human patients [77, 78]. Male babies are at higher risk of cerebral palsy than females [79]. Not only are motor deficits significantly more severe in male infants [77], but structural magnetic resonance imaging (MRI) has demonstrated a qualitatively different pattern of injury in males and females. One study reported that white-matter injury patterns predominated in male babies, whereas females were more likely to demonstrate a grey-matter injury pattern [80]. These relatively recent discoveries led to reanalysis of a clinical trial of prostaglandin inhibitor indomethacin as a preventative treatment in infants at high-risk of intraventricular haemorrhage [77, 81]. When cognitive and motor development were assessed in a mixed-sex group at age 3, there was no difference between treated and untreated groups. However, when boys and girls were analysed separately, the anti-inflammatory drug improved functionality in boys given indomethacin compared to boy who did not receive treatment. New contributing factors for hypoxic ischaemia injury continue to be uncovered, and this surprising revelation reinforces the argument that our current models should remain under revision.

The physiological basis of this sexual dimorphism remains poorly understood [16, 77, 78]. Neuronal culture models have identified sex-specific differences in cell death cascades induced by hypoxia *in vitro*. One of the first studies to suggest a molecular basis for this sexual dimorphism cultured XX (female) and XY (male) neurons separately and triggered neuronal cell death by administering nitric oxide (NO) and glutamate [82]. The mitochondria of male and female neurons released different molecules, and the male neurons were less able to maintain antioxidant expression. This finding has been expanded upon considerably since. The putative treatment 2-iminobiotin appears to have different effects on neurons from male and female rats [83]. In males, there was no significant effect, whereas female rats showed decreased activation of the cytochrome C cascpase-3 pathway, and its downstream cell death markers. Another investigation found that female neonatal rats expressed greater levels of cleaved caspase-3, the activated form of an important cell death promoting molecule, than male brains, although there was no difference between the sexes in nitrotyrosine or autophagy [84].

Sexual dimorphism in neonatal hypoxia ischaemia is receiving increasing attention. This is an expanding area of research, with recent *in vivo* studies uncovering unexpected results. There is now robust evidence that the increased vulnerability seen in male human patients extends to rodents [77, 84]. When equivalent procedures were used to generate hypoxia ischaemia in rats over a range of developmental stages, the only significant difference in lesion outcome

between the sexes was detected at a perinatal age, with no difference in older rat pups or fully-developed adults [84]. Animal models of neonatal HI support a fundamental difference in mitochondrial respiratory function in the developing male and female brain [85, 86], with female mitochondria posited as more resilient. Mitochondrial function may not fully explain the difference between the sexes, as evidence is now emerging that drugs targeting neurotransmitter receptors may only be effective in one sex, males [87]. Sex hormone therapy, such as progesterone treatment, is protective against HI injury in male rats but had no effect in females [78, 88]. Despite suggestions that the adult brain's response to ischaemic stroke may also be sex-dependent [77], the evidence currently suggests that this difference is largely a neonatal phenomenon.

3. Treatments of neonatal hypoxia ischaemia

Despite the high rates of disability in human survivors of neonatal hypoxia ischaemia [4, 16], only one treatment is currently licenced in the UK: hypothermia. This therapy reduces the infant's body or head to approximately 33°C [16, 89]. Hypothermia was first demonstrated to improve survival in cases of cardiac arrest [90], and has since been applied as a neuroprotective treatment in acute neonatal hypoxia ischaemia patients [89, 91]. One meta-analysis of over 1200 infants found that hypothermia reduced death and neurological handicaps at 18 months follow-up across all severities of neonatal hypoxia ischaemia [89].

However, hypothermia alone is not sufficient to prevent all brain injury or neurological symptoms [4, 16, 89]. Since the discovery of the neuroprotective effect of hypothermia, little progress has been made towards additive therapies. Few potential treatments have reached clinical trials. The development of novel treatments to supplement hypothermia is imperative. In this section, current research into novel treatments for neonatal hypoxia ischaemia will be reviewed and approaches for therapy development will be evaluated.

3.1. Review of therapies under development for infant hypoxia ischaemia

Two additional interventions have been deemed safe to trial in neonates. Resuscitation at room temperature [92] and xenon gas administration [93] have been investigated in a clinical environment alongside hypothermia. The limited success reported in these studies is now under speculation. Recent randomised clinical trials have demonstrated that although xenon gas is a safe treatment, there is little or no therapeutic effect of combined hypothermia and xenon gas in moderate and severe cases of neonatal HI at 18 months follow-up [94]. A parallel experiment in rats found that xenon made no difference to lesion size or neuronal cell numbers in cases of severe hypoxia ischaemia [95].

Pharmacological agents have also been investigated in human neonatal patients, resulting in limited success. Barbiturate anticonvulsants had no effect on long-term neurological development when given to hypoxic ischaemic neonates [96]. A more promising result from recent clinical studies suggests that high-dose erythropoietin (EPo) treatment in term neonates reduces disability [97]. However, even proponents of this potential treatment advise caution

in interpreting early results. The therapy does not completely prevent neurological symptoms. There is hope for erythropoietin as a future additive treatment, yet the field should be concerned that this is currently the only pharmacological molecule being pursued in clinical trials for neonatal hypoxia ischaemia.

Many more small molecules are being investigated in animal models of neonatal hypoxia ischaemia, where the translational value of the research, and the safety of the treatment in vulnerable newborns, remain uncertain. For example, free radical scavenger N-acetylcysteine and systemic hypothermia reduced infarct volume after focal hypoxic ischaemic injury in rats [98, 99]. Another free radical scavenger, allopurinol, reduced cerebral oedema and neuropathological damage [99, 100].

One example of the difficulty involved in selecting targets for new therapies is the lack of clinical translation of the extensive work on NMDA receptor-mediated excitotoxicity in the neonatal hypoxic ischaemic brain. Drugs that block NMDA receptors are protective against HI injury in neonatal rodent models [101]. Despite the efficacy of NMDA receptor antagonists in reducing infarct volumes in rats, this work has not been pursued in humans as intact NMDA-mediated classical neuronal plasticity is essential for normal brain development [16, 102, 103]. Effective NMDA antagonists could cause more damage to the circuitry of the neonatal brain than is justified by their anti-excitotoxicity function, undermining the medical philosophy enshrined in the Hippocratic oath: to do no harm.

There are several novel treatments being developed by dedicated scientists, although it is extremely difficult to predict which of these will be deemed safe enough to allow clinical trials in the developing brain. Perhaps the translation from bench to bedside for putative treatments could be improved through a different approach to treatment selection and funding. Some essential factors demanding consideration at the earliest point in treatment development are outlined below.

3.2. Proposed approaches to therapy development for infant hypoxia ischaemia

New approaches are required to identify potential treatments for neonatal hypoxia ischaemia which will be better suited to advance into clinical trials. Three essential properties must be satisfied in a new therapy. These have been suggested in a previous publication I authored [16], and are summarised in **Figure 3**. First, all potential treatments should be safe for vulnerable neonates and not interfere with essential developmental milestones. Second, treatments should be specific, to avoid extreme adverse effects in vulnerable infants. And third, an ideal treatment would target molecules common to the excitotoxicity, oxidative stress, and inflammation pathways. Targeting common mediators would allow a single therapy to be efficacious against multiple mechanisms of brain damage, instead of merely eliciting a reshuffle to favour a different method of cell death [62, 102]. These three qualities can be summarised as safety, specificity, and breadth.

The suitability of any future small molecules for use in human neonates will depend greatly on the severity of any adverse effects on brain development. A wide range of molecules contribute to healthy brain development in the neonatal period [16, 103, 104], a time of widespread

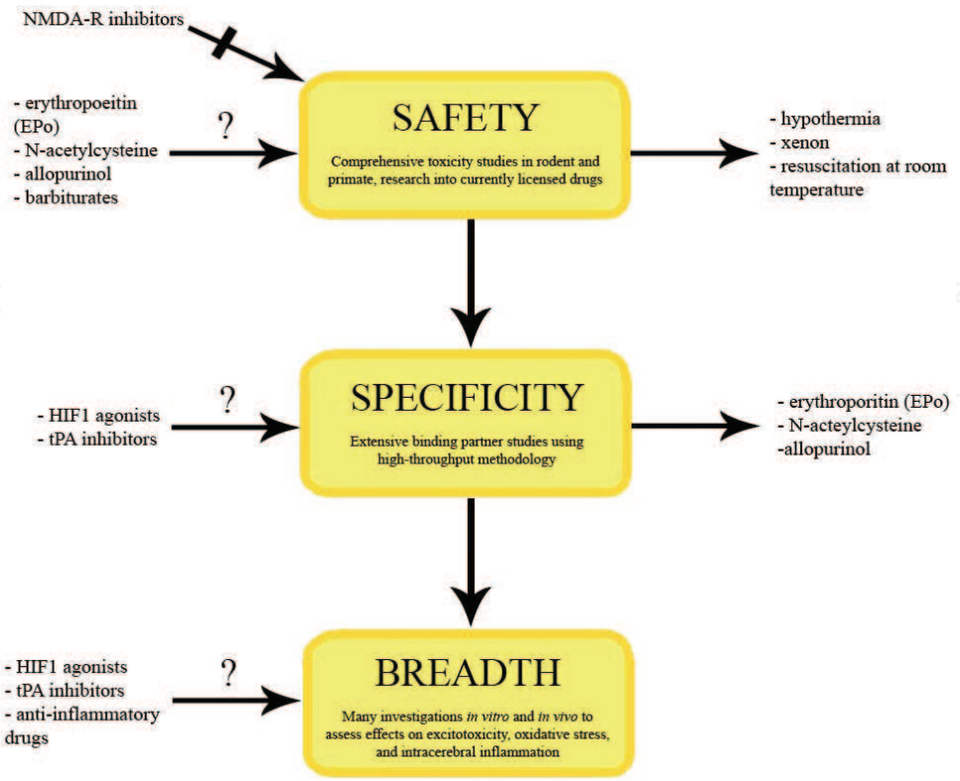

Figure 3. Schematic depiction of proposed filters for putative therapies for infant hypoxia ischaemia. All putative treatments should satisfy safety prior to investigation in human infants. Specificity may also decrease off-target effects, and breadth may increase treatment efficacy. Several current treatments are listed by which criteria these satisfy. HIF1 = hypoxia inducible factor 1, tPA = tissue plasminogen activator, NMDA-R = *N*-methyl-D-aspartate receptor, EPo = erythropoietin.

remodelling and plasticity within the brain. Selecting molecular targets known to be expressed in the neonatal brain could reduce the chances of general toxicity, but does not preclude the possibility that endogenous proteins may have a narrow therapeutic range, with slight increases or decreases interfering with development. Future studies of potential treatments should examine developmental plasticity processes, to ensure safety in the neonatal brain.

Specificity is also a desired characteristic of any potential pharmacological therapy for neonates, as faithfulness of a single molecular target minimises the likelihood of off-target side-effects. The cardiovascular and respiratory systems of neonates are vulnerable in premature birth and following hypoxic ischaemia injury [5, 8], so brain-specific neuroprotective treatments are desirable. Molecular specificity is essential in addition to organ specificity. Extensive characterisation of the binding partners of not only the pharmacological molecule, but also its biological molecular target, is time-consuming but essential work if a therapy is to be estimated as safe enough for trial in human neonates. As high-throughput screening methods are increasingly refined for use throughout the pharmaceutical industry [105, 107], capturing specificity is becoming a realistic research goal.

Breadth of action is essential for the efficacy of a therapy designed for neonatal hypoxia ischaemia, in which a wide range of neuronal death pathways are active simultaneously in

the injured brain. These cascades, which include excitotoxicity, oxidative stress, and inflammation, are not entirely independent of one another. It may be possible to identify a "master regulator". This hypothetical single molecule would dampen multiple brain damage pathways, inhibiting a key activator (or activators) of each respective process, and perhaps trigger other neuroprotective cascades. But how probable is it that a "master regulator" will be discovered? Is it possible that one has already been documented and simply remains to be exploited?

The concept of a "master regulator" for any complex disease appears enticing, but is its promise only linguistic trickery? Identification of candidate proteins will not be a simple process, likely requiring many experiments spanning multiple methods. One possible starting point is microarray data collected following neonatal hypoxia ischaemia in rodents [106, 107]. Microarrays are highly sensitive to the time of tissue collection post-injury, and do not detect changes in functional protein content mediated by translational modification or secretion. For example, no published microarrays detected changes in tissue plasminogen activator (tPA) or hypoxia inducible factor 1 (HIF1) transcription, although these proteins play a substantial role in injury pathogenesis [108, 109]. Generating a neonatal hypoxia ischaemia 'secretome' [110] could help identify the earliest changes in protein activity directly following neonatal brain injury.

Some proteins are already known to span multiple cell death cascades [16, 62, 102]. These are clearly the most accessible candidates for "master regulator" properties. NMDA receptors, major mediators of neuronal death by excitotoxicity, can be directly or indirectly activated by free radicals, combining two lethal molecular cascades often treated as separate in the literature [16, 25, 47]. Inflammatory pathways also mediate excitotoxicity and oxidative stress. In rodents, pre-treatment with IL-1ß, IL-6, IL-9, or TNF-α enhances brain damage caused by NMDA agonists [16, 64, 73]. It is these overlaps between cascades at which a "master regulator" could act. One candidate is HIF1 [109]. This transcription factor is known to regulate a minimum of 60 genes, including the putative therapeutic molecule erythropoietin, several growth factors, and mitochondrial proteins. Another possible "master regulator", tissue plasminogen activator, is currently one of the best-documented possibilities [16, 108]. tPA has established roles crossing boundaries between excitotoxicity, oxidative stress, and brain inflammation. The relative dearth of candidates proposed here perhaps reflects our incomplete knowledge of the molecular mechanisms underpinning neonatal hypoxic ischaemic brain damage. There is no clear single "master regulator" protein documented in the literature of this complex neurodevelopmental disorder. However, this does not restrain future experimenters from seizing on those few currently supported candidates for further development.

4. Conclusions

As the most common single cause of infant mortality and morbidity globally, neonatal and perinatal hypoxia ischaemia deserve wide recognition and funding within the research community. These conditions require careful examination in animal models closely matched to the

level of brain development at birth in humans, as there is a plethora of differences between the adult and infant brain which create infant-specific challenges for understanding neuropathology and developing new therapies. Infant-specific obstacles also exist, as any treatment should not interfere with normal brain development, or the vulnerable infant cardiovascular system. Despite these constraints, it should be possible to develop novel therapeutics closely guided by the criteria of safety, specificity, and breadth. Current research has suggested some promising candidate neuroprotective treatments, such as erythropoietin and tissue plasminogen activator, and these could yet inform future approaches to therapeutic development.

Acknowledgements

I would like to thank my DPhil supervisor Professor Zoltán Molnár and senior researcher Dr. Anna Hoerder-Suabedissen for their enormous contributions towards the successful completion of my doctorate. I would also like to thank Professor Pierre Gressens, Professor David Edwards, Dr. Bobbi Fleiss, Dr. Claire Thornton, and Dr. Ana Baburamani from King's College London for educating me about neonatal hypoxia ischaemia. And finally, my wonderful partner Adam Barker for all the support he gives me.

Conflict of interest

The author declares that the review article was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author details

Lancelot Jamie Millar

Address all correspondence to: lancelot.millar@univ.ox.ac.uk

Department of Physiology, Anatomy, and Genetics (DPAG), University of Oxford, Oxford, United Kingdom

References

[1] Perlman JM. Intrapartum hypoxic-ischemic cerebral injury and subsequent cerebral palsy: Medicolegal issues. Pediatrics. 1997;**99**:851-859

[2] Perlman JM. Summary proceedings from the neurology group on hypoxic-ischemic encephalopathy. Pediatrics. 2006;**117**:S28-S33

[3] Volpe JJ. Hypoxic-Ischemic Encephalopathy in Neurology of the Newborn. Philadelphia, PA: WB Saunders; 2001

[4] Volpe JJ. Neonatal encephalopathy: An inadequate term for hypoxic–ischemic encephalopathy. Annals of Neurology. 2012;**72**:156-166

[5] Finer N, Robertson C, Richards R, et al. Hypoxic-ischemic encephalopathy in term neonates: Perinatal factors and outcome. The Journal of Pediatrics. 1981;**98**:112-117

[6] Richer LP, Shevell MI, Miller SP. Diagnostic profile of neonatal hypotonia: An 11-year study. Pediatric Neurology. 2001;**25**:32-37

[7] Levene M, Grindulis H, Sands C, et al. Comparison of two methods of predicting outcome in perinatal asphyxia. Lancet. 1986;**327**:67-69

[8] Richardson BS, Carmichael L, Homan J, et al. Fetal cerebral, circulatory, and metabolic responses during heart rate decelerations with umbilical cord compression. American Journal of Obstetrics and Gynecology. 1996;**175**(4):929-936

[9] Ruth VJ, Raivio KO. Perinatal brain damage: Predictive value of metabolic acidosis and the Apgar score. BMJ. 1988;**297**:24-27

[10] Robertson C, Finer N, Grace M. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. The Journal of Pediatrics. 1989;**114**: 753-760

[11] Volpe JJ, editor. Hypoxic-ischemic encephalopathy: Neuropathology and pathogenesis. In: Neurology of the Newborn. London: W B Saunders; 1995. pp. 279-313

[12] Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. Nature Reviews. Neurology. 2015;**11**:192-208

[13] Grow J, Barks JD. Pathogenesis of hypoxic-ischemic cerebral injury in the term infant: Current concepts. Clinics in Perinatology. 2002;**29**:585-602

[14] Ferriero DM. Neonatal brain injury. The New England Journal of Medicine. 2004;**351**: 1985-1995

[15] Shalak L, Perlman JM. Hypoxic–ischemic brain injury in the term infant-current concepts. Early Human Development. 2004;**80**:125-141

[16] Millar LJ, Hoerder-Suabedissen A, Shi L, et al. Neonatal hypoxia Ischaemia: Mechanisms, models, and therapeutic challenges. Frontiers in Cellular Neuroscience. 2017;**8**: 11-78

[17] Graham EM, Ruis KA, Hartman AL, et al. A systematic review of the role of intrapartum hypoxia-ischemia in the causation of neonatal encephalopathy. American Journal of Obstetrics and Gynecology. 2008;**199**:587-595

[18] Dilenge ME, Majnemer A, Shevell MI. Long-term developmental outcome of asphyxiated term neonates. Journal of Child Neurology. 2001;**16**:781-792

[19] De Souza SW, Richards B. Neurological sequelae in newborn babies after perinatal asphyxia. Archives of Disease in Childhood. 1978;**53**:564-569

[20] Lee AC, Kozuki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatric Research. 2013;**74**:50-72

[21] Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: When? Where? Why? Lancet. 2005;**365**:891-900

[22] Vincer MJ, Allen AC, Joseph KS, et al. Increasing prevalence of cerebral palsy among very preterm infants: A population-based study. Pediatrics. 2006;**118**:e1621-e1626

[23] Robertson NJ, Iwata O. Bench to bedside strategies for optimizing neuroprotection following perinatal hypoxia–ischaemia in high and low resource settings. Early Human Development. 2007;**83**:801-811

[24] Wilson-Costello D, Friedman H, Minich N, et al. Improved neurodevelopmental outcomes for extremely low birth weight infants in 2000-2002. Pediatrics. 2007;**119**:37-45

[25] Baburamani AA, Ek CJ, Walker DW, et al. Vulnerability of the developing brain to hypoxic-ischemic damage: Contribution of the cerebral vasculature to injury and repair? Frontiers in Physiology. 2012;**3**:424

[26] Volpe JJ. Intraventricular hemorrhage in the premature infant–current concepts. part I. Annals of Neurology. 1989;**25**:3-11

[27] Ment LR, Stewart WB, Ardito TA, et al. Beagle pup germinal matrix maturation studies. Stroke. 1991;**22**:390-395

[28] Perlman D, Halvorson HO. A putative signal peptidase recognition site and sequence in eukaryotic and prokaryotic signal peptides. Journal of Molecular Biology. 1983;**167**(2): 391-409

[29] Greisen G, Johansen K, Ellison PH, et al. Cerebral blood flow in the newborn infant: Comparison of Doppler ultrasound and 133xenon clearance. The Journal of Pediatrics. 1984;**104**(3):411-418

[30] Younkin DP, Reivich M, Jaggi JL, et al. The effect of hematocrit and systolic blood pressure on cerebral blood flow in newborn infants. Journal of Cerebral Blood Flow & Metabolism. 1987;**7**(3):295-299

[31] Wigglesworth JS, Pape KE. An integrated model for haemorrhagic and ischaemic lesions in the newborn brain. Early Human Development. 1978;**2**(2):179-199

[32] El-Khoury N, Braun A, Hu F, et al. Astrocyte end-feet in germinal matrix, cerebral cortex, and white matter in developing infants. Pediatric Research. 2006;**59**:673-679

[33] Lou HC, Lassen NA, Friis-Hansen B. Impaired autoregulation of cerebral blood flow in the distressed newborn infant. The Journal of Pediatrics 1979. 1979;**94**(1):118-121

[34] Tsuji M, Saul JP, du Plessis A, et al. Cerebral intravascular oxygenation correlates with mean arterial pressure in critically ill premature infants. Pediatrics. 2000;**106**(4):625-632

[35] Pryds O, Greisen G, Lou H, et al. Vasoparalysis associated with brain damage in asphyxiated term infants. The Journal of Pediatrics. 1990;**117**(1):119-125

[36] Hardy P, Nuyt AM, Dumont I, et al. Developmentally increased cerebrovascular NO in newborn pigs curtails cerebral blood flow autoregulation. Pediatric Research. 1999;**46**: 375-382

[37] Muramatsu K, Fukuda A, Togari H, et al. Vulnerability to cerebral hypoxic-ischemic insult in neonatal but not in adult rats is in parallel with disruption of the blood-brain barrier. Stroke. 1997;**28**:2281-2289

[38] Stonestreet BS, Sadowska GB, Leeman J, et al. Effects of acute hyperosmolality on blood-brain barrier function in ovine fetuses and lambs. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2006;**291**(4):R1031-R1039

[39] Svedin P, Hagberg H, Savman K, et al. Matrix metalloproteinase-9 gene knock-out protects the immature brain after cerebral hypoxia-ischemia. The Journal of Neuroscience. 2007;**27**:1511-1518

[40] Tu YF, Tsai YS, Wang LW, et al. Overweight worsens apoptosis, neuroinflammation and blood-brain barrier damage after hypoxic ischemia in neonatal brain through JNK hyperactivation. Journal of Neuroinflammation. 2011;**8**:40

[41] Yang D, Li SY, Yeung CM, et al. *Lycium barbarum* extracts protect the brain from blood-brain barrier disruption and cerebral edema in experimental stroke. PLoS One. 2012;**7**: e33596

[42] Stolp HB, Dziegielewska KM, Ek CJ, et al. Breakdown of the blood–brain barrier to proteins in white matter of the developing brain following systemic inflammation. Cell and Tissue Research. 2005;**320**(3):369-378

[43] Stolp HB, Johansson PA, Habgood MD, et al. Effects of neonatal systemic inflammation on blood-brain barrier permeability and behaviour in juvenile and adult rats. Cardiovascular Psychiatry and Neurol. 2011;**2011**:469046

[44] Ek CJ, Habgood MD, Dziegielewska KM, et al. Structural characteristics and barrier properties of the choroid plexuses in developing brain of the opossum (*Monodelphis domestica*). The Journal of Comparative Neurology. 2003;**460**:451-464

[45] Ek CJ, Dziegielewska KM, Stolp H, et al. Functional effectiveness of the blood-brain barrier to small water-soluble molecules in developing and adult opossum (*Monodelphis domestica*). The Journal of Comparative Neurology. 2006;**496**:13-26

[46] Stonestreet BS, Burgess GH, Cserr HF. Blood-brain barrier integrity and brain water and electrolytes during hypoxia/hypercapnia and hypotension in newborn piglets. Brain Research. 1992;**590**:263-270

[47] Hagberg H, Andersson P, Kjellmer I, et al. Extracellular overflow of glutamate, aspartate, GABA and taurine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia. Neuroscience Letters. 1987;**78**:311-317

[48] Tremblay E, Roisin MP, Represa A, et al. Transient increased density of NMDA binding sites in the developing rat hippocampus. Brain Research. 1988;**461**:393-396

[49] Danysz W, Parsons CG. Glycine and N-methyl-D-aspartate receptors: Physiological significance and possible therapeutic applications. Pharmacological Reviews. 1998;**50**:597-664

[50] McDonald JW, Silverstein FS, Johnston MV. Neurotoxicity of N-methyl-D-aspartate is markedly enhanced in developing rat central nervous system. Brain Research. 1988;**459**: 200-203

[51] Pu Y, Garg A, Corby R, et al. A positive correlation between alpha-glutamate and glutamine on brain 1H-MR spectroscopy and neonatal seizures in moderate and severe hypoxic-ischemic encephalopathy. AJNR. American Journal of Neuroradiology. 2008;**29**: 216

[52] Holmes GL, Ben-Ari Y. The neurobiology and consequences of epilepsy in the developing brain. Pediatric Research. 2001;**49**:320-325

[53] Rakhade SN, Jensen FE. Epileptogenesis in the immature brain: Emerging mechanisms. Nature Reviews. Neurology. 2009;**5**:380-391

[54] Williams PA, Dou P, Dudek FE. Epilepsy and synaptic reorganization in a perinatal rat model of hypoxia-ischemia. Epilepsia. 2004;**45**:1210-1218

[55] Ferriero DM. Oxidant mechanisms in neonatal hypoxia-ischemia. Developmental Neuroscience. 2001;**23**:198-202

[56] Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. Clinics in Perinatology. 2009;**36**:835-837

[57] Lizasoain I, Weiner CP, Knowles RG, Moncada S. The ontogeny of cerebral and cerebellar nitric oxide synthase in the Guinea pig and rat. Pediatric Research. 1996;**39**(5):779

[58] Baud O, Greene AE, Li J, et al. Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. The Journal of Neuroscience. 2004;**24**(7):1531-1540

[59] Fullerton HJ, Ditelberg JS, Chen SF, et al. Copper/zinc superoxide dismutase transgenic brain accumulates hydrogen peroxide after perinatal hypoxia ischemia. Annals of Neurology. 1998;**44**(3):357-364

[60] Stiller R, von Mering R, Konig V, et al. How well does reflectance pulse oximetry reflect intrapartum fetal acidosis? American Journal of Obstetrics and Gynecology. 2002;**186**: 1351-1357

[61] O'Brien JS, Sampson EL. Myelin membrane: A molecular abnormality. Science. 1965;**150**: 1613-1614

[62] Northington FJ, Ferriero DM, Graham EM, et al. Early neurodegeneration after hypoxia-ischemia in neonatal rat is necrosis while delayed neuronal death is apoptosis. Neurobiology of Disease. 2001;**8**:207-219

[63] Blomgren K, Hagberg H. Free radicals, mitochondria, and hypoxia-ischemia in the developing brain. Free Radical Biology & Medicine. 2006;**40**:388-397

[64] Dean JM, Shi Z, Fleiss B, et al. A critical review of models of perinatal infection. Developmental Neuroscience. 2015;**37**:289-304

[65] Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA. 2004; **292**:2357-2365

[66] Mitha A, Foix-L'Helias L, Arnaud C, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. Pediatrics. 2013;**132**:e372-e380

[67] Lehnardt S, Massillon L, Follett P, et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**:8514-8519

[68] Eklind S, Mallard C, Arvidsson P, et al. Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. Pediatric Research. 2005;**58**: 112-116

[69] Agrawal V, Hirsch E. Intrauterine infection and preterm labor. Seminars in Fetal & Neonatal Medicine. 2012;**17**:12-19

[70] Wang X, Hagberg H, Zhu C, et al. Effects of intrauterine inflammation on the developing mouse brain. Brain Research. 2007;**1144**:180-185

[71] Duncan JR, Cock ML, Scheerlinck JP, et al. White matter injury after repeated endotoxin exposure in the preterm ovine fetus. Pediatric Research. 2002;**52**:941-949

[72] Tahraoui SL, Marret S, Bodenant C, et al. Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter. Brain Pathology. 2001;**11**:56-71

[73] Dommergues M, Plaisant F, Verney C, et al. Early microglial activation following neonatal excitotoxic brain damage in mice: A potential target for neuroprotection. Neuroscience. 2003;**121**:619-628

[74] Mallard C, Davidson JO, Tan S, et al. Astrocytes and microglia in acute cerebral injury underlying cerebral palsy associated with preterm birth. Pediatric Research. 2013;**75**:234-240

[75] Kaur C, Rathnasamy G, Ling E. Roles of activated microglia in hypoxia induced neuroinflammation in the developing brain and the retina. Journal of Neuroimmune Pharmacology. 2013;**8**:66-78

[76] Faustino JV, Wang X, Johnson CE, et al. Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke. The Journal of Neuroscience. 2011;**31**: 12992-13001

[77] Johnston MV, Hagberg H. Sex and the pathogenesis of cerebral palsy. Developmental Medicine & Child Neurology. 2007;**49**(1):74-78

[78] Hill CA, Fitch RH. Sex differences in mechanisms and outcome of neonatal hypoxia-ischemia in rodent models: Implications for sex-specific neuroprotection in clinical neonatal practice. Neurology Research International. 2012;**2012**:867531

[79] Jarvis S, Glinianaia SV, Arnaud C, et al. Case gender and severity in cerebral palsy varies with intrauterine growth. Archives of Disease in Childhood. 2005;**90**:474-479

[80] Thompson DK, Warfield SK, Carlin JB, et al. Perinatal risk factors altering regional brain structure in the preterm infant. Brain. 2007;**130**(3):667-677

[81] Ment LR, Vohr BR, Makuch RW, et al. Prevention of intraventricular hemorrhage by indomethacin in male preterm infants. The Journal of Pediatrics. 2004;**145**(6):832-834

[82] Du L, Hickey RW, Bayir H, et al. Starving neurons show sex difference in autophagy. The Journal of Biological Chemistry. 2009;**284**:2383-2396

[83] Nijboer CH, Groenendaal F, Kavelaars A, et al. Gender-specific neuroprotection by 2-iminobiotin after hypoxia-ischemia in the neonatal rat via a nitric oxide independent pathway. Journal of Cerebral Blood Flow and Metabolism. 2007;**27**:282-292

[84] Zhu C, Xu F, Wang X, et al. Different apoptotic mechanisms are activated in male and female brains after neonatal hypoxia-ischaemia. Journal of Neurochemistry. 2006;**96**: 1016-1027

[85] Demarest TG, Shuh RA, Waddell J, et al. Sex-dependent mitochondrial respiratory impairment and oxidative stress in a rat model of neonatal hypoxic-ischemic encephalopathy. Journal of Neurochemistry. 2016;**137**(5):714-729

[86] Demarest TG, Shuh RA, Waite EL, et al. Sex dependent alterations in mitochondrial electron transport chain proteins following neonatal rat cerebral hypoxic-ischemia. Journal of Bioenergetics and Biomembranes. 2016;**48**(6):591-598

[87] Gillani QA, Akbar A, Ali M, et al. Gender-specific effects of CGP 55845, $GABA_B$ receptor antagonist, on neuromuscular coordination, learning and memory formation in albino mouse following neonatal hypoxia–ischemia insult. Journal of the Neurological Sciences. 2015;**36**(6):961-969

[88] Peterson BL, Won S, Gebees RI, et al. Sex-related differences in effects of progesterone following neonatal hypoxic brain injury. Behavioural Brain Research. 2015;**268**:152-168

[89] Tagin MA, Woolcott CG, Vincer MJ, et al. Hypothermia for neonatal hypoxic ischemic encephalopathy: An updated systematic review and meta-analysis. Archives of Pediatrics & Adolescent Medicine. 2012;**166**:558-566

[90] Bernard SA, Gray TW, Buist MD, et al. Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. The New England Journal of Medicine. 2002;**346**:557-563

[91] Gunn AJ, Battin M, Gluckman PD, et al. Therapeutic hypothermia: From lab to NICU. Journal of Perinatal Medicine. 2005;**33**:340-346

[92] Rabi Y, Rabi D, Yee W. Room air resuscitation of the depressed newborn: A systematic review and meta-analysis. Resuscitation. 2007;**72**:353-363

[93] Thoresen M, Hobbs CE, Wood T, et al. Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia. Journal of Cerebral Blood Flow and Metabolism. 2009;**29**:707-714

[94] Azzopardi D, Robertson NJ, Bainbridge A, 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 Neurology. 2015;**15**(2):145-153

[95] Sabir H, Osredkar D, Maes E, et al. Xenon combined with therapeutic hypothermia is not neuroprotective after severe hypoxia-ischemia in neonatal rats. PLoS One. 2016;**11**: e0156759

[96] Evans DJ, Levene MI, Tsakmakis M. Anticonvulsants for preventing mortality and morbidity in full term newborns with perinatal asphyxia. Cochrane Database of Systematic Reviews. 2007;**3**:CD001240

[97] McPherson RJ, Juul SE. Erythropoietin for infants with hypoxic-ischemic encephalopathy. Current Opinion in Pediatrics. 2010;**22**:139-145

[98] Jatana M, Singh I, Singh AK, et al. Combination of systemic hypothermia and N-acetylcysteine attenuates hypoxic-ischemic brain injury in neonatal rats. Pediatric Research. 2006;**59**:684-689

[99] Lai MC, Yang SN. Perinatal hypoxic-ischemic encephalopathy. Journal of Biomedicine & Biotechnology. 2011;**2011**:609813

[100] Palmer C, Vannucci RC, Towfighi J. Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. Pediatric Research. 1990;**27**(4):332-336

[101] McDonald JW, Silverstein FS, Johnston MV. Neuroprotective effects of MK-801, TCP, PCP and CPP against N-methyl-D-aspartate induced neurotoxicity in an *in vivo* perinatal rat model. Brain Research. 1989;**490**:33-40

[102] Northington FJ, Chavez-Valdez R, Martin LJ. Neuronal cell death in neonatal hypoxia-ischemia. Annals of Neurology. 2011;**69**:743-758

[103] Ikonomidou C, Bosch F, Miksa M, et al. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science. 1999;**283**:70-74

[104] Rocha-Ferreira E, Hristova M. Antimicrobial peptides and complement in neonatal hypoxia-ischemia induced brain damage. Frontiers in Immunology. 2015;**6**:56

[105] Sundberg SA. High-throughput and ultra-high-throughput screening: Solution-and cell-based approaches. Current Opinion in Biotechnology. 2000;**11**(1):47-53

[106] Carmel JB, Kakinohana O, Mestril R, et al. Mediators of ischemic preconditioning identified by microarray analysis of rat spinal cord. Experimental Neurology. 2004;**185**:81-96

[107] Rognlien AGW, Wollen EJ, Atneosen-Åsegg M, et al. Increased expression of inflammatory genes in the neonatal mouse brain after hyperoxic reoxygenation. Pediatric Research. 2014;**77**:326-333

[108] Henry VJ, Lecointre M, Laudenbach V, et al. High tPA release by neonate brain microvascular endothelial cells under glutamate exposure affects neuronal fate. Neurobiology of Disease. 2013;**50**:201-208

[109] Sheldon RA, Osredkar D, Lee CL, et al. HIF-1α-deficient mice have increased brain injury after neonatal hypoxia-ischemia. Developmental Neuroscience. 2009;**31**(5):452-458

[110] Tjalsma H, Antelmann H, Jongbloed JD, et al. Proteomics of protein secretion by *Bacillus subtilis*: Separating the "secrets" of the secretome. Microbiology and Molecular Biology Reviews. 2004;**68**(2):207-233