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

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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 in 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 CO₂ 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.

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 CO₂ 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 (IL1 β , IL6), tumour necrosis factor alpha (TNF α), 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 O₂ 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

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 caspase-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-1 β , 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.

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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.

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