Ventilation-Induced Brain Injury in Preterm Neonates: A Review of Potential Therapies

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Key Words

Ventilation · Neonate · Delivery room · Brain · Resuscitation · Therapy

Abstract

Mechanical ventilation is a risk factor for cerebral inflammation and brain injury in preterm neonates. The risk increases proportionally with the intensity of treatment. Recent studies have shown that cerebral inflammation and injury can be initiated in the delivery room. At present, initiation of intermittent positive pressure ventilation (IPPV) in the delivery room is one of the least controlled interventions a preterm infant will likely face. Varying pressures and volumes administered shortly after birth are sufficient to trigger pathways of ventilation-induced lung and brain injury. The pathways involved in ventilation-induced brain injury include a complex inflammatory cascade and haemodynamic instability, both of which have an impact on the brain. However, regardless of the strategy employed to deliver IPPV, any ventilation has the potential to have an impact on the immature brain. This is particularly important given that preterm infants are

already at a high risk for brain injury simply due to immaturity. This highlights the importance of improving the initial respiratory support in the delivery room. We review the mechanisms of ventilation-induced brain injury and discuss the need for, and the most likely, current therapeutic agents to protect the preterm brain. These include therapies already employed clinically, such as maternal glucocorticoid therapy and allopurinol, as well as other agents, such as erythropoietin, human amnion epithelial cells and melatonin, already showing promise in preclinical studies. Their mechanisms of action are discussed, highlighting their potential for use immediately after birth.

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Preterm Birth, Brain Injury and Ventilation Requirement

Preterm birth, defined as birth prior to 37 completed weeks of gestation and with an incidence of 7–12% worldwide [1], results in the birth of neonates with underdeveloped lungs. As a result, 92% of extremely preterm infants

(born at <28 weeks of gestation) in Australia and New Zealand require some form of assisted ventilation for at least 4 h after birth [2]; for many, this ventilation can occur as early as in the delivery room [3]. Whilst this respiratory assistance is essential for survival, ventilation can increase the risk and incidence of brain injury. Given that extremely preterm infants are already at an elevated risk for brain injury, any intervention applied perinatally, including the initiation of respiratory support, needs to be optimized.

Unlike for the neonatal intensive care unit (NICU), there is a lack of guidelines and protocols to inform the initiation of ventilation in the delivery room and often an absence of sophisticated devices [4, 5]. As a consequence, more than 85% of preterm infants who receive assisted ventilation in the delivery room are given dangerously high tidal volumes (V_T) [6], with potential detrimental effects on the lungs [7, 8] and brain [9, 10]. A small study of preterm infants showed an increased incidence of intraventricular haemorrhage (IVH) in babies receiving a high V_T compared to babies receiving a low V_T in the delivery room [11]. Thus, whilst it is well established that prolonged mechanical ventilation can be injurious to the preterm lungs and brain, ventilation-induced injury may occur as early as the initiation of ventilation in the delivery room and seems to be a potentially preventable contributor to brain injury.

The use of intermittent positive pressure ventilation (IPPV) in the delivery room is decreasing as non-invasive ventilation strategies, such as continuous positive-airway pressure, are increasingly introduced. However, despite the reduction in the number of infants requiring intubation and/or IPPV, this has not translated into improved neurological outcomes [12]. Mechanisms responsible for brain injury arising from IPPV have been identified via animal experimentation and reviewed in detail previously [13], and hence this review will focus on IPPV. Mechanistic investigations of brain injury associated with non-invasive ventilation strategies are lacking, but we anticipate that the underlying causes are similar to those elicited by IPPV.

The first pathway to brain injury from ventilation is the initiation of a pulmonary inflammatory cascade [8] which migrates systemically to the brain, where it can cause a localized cerebral inflammatory response sufficient to increase markers of oxidative stress and apoptosis [9, 10, 14]. The second pathway is caused by over-distension of alveoli and compression of pulmonary capillaries, increasing pulmonary resistance and decreasing the cardiac output [15]. Coupled with immature autoregulation and a permeable blood-brain barrier, this haemodynam-

ic disturbance causes variable blood flow to the brain and cerebral protein extravasation [10, 14]. These pathways are amplified when the initiation of ventilation encompasses a high V_T [10]. Whilst improving ventilation strategies can minimize some aspects of ventilation-induced lung and brain injury, it is not sufficient to mitigate injury [10, 16]. Thus, IPPV, irrespectively of the strategy, can increase brain injury and inflammation from as early as its initiation.

Similarly to ventilation, cerebral inflammation and cerebral haemodynamic instability are also two of the critical pathways involved in preterm brain injury [17, 18]. Thus, a preterm neonate will already be vulnerable to these pathways and a subsequent intervention, such as ventilation, can exacerbate these pathways. It is imperative that we identify an adjunct therapy that has the potential to target these two critical pathways. This will ultimately reduce ventilation-induced brain injury as well as provide protection to the already vulnerable preterm brain. An ideal therapeutic would stabilize cardiopulmonary-cerebral haemodynamics during the transition at birth and mitigate the cerebral inflammatory response. Medical databases (PubMed, EMBASE and Google Scholar) were screened, and results were limited to the English language, to review the neuroprotective benefits of current clinical antenatal treatments, i.e. maternal glucocorticosteroid therapy and allopurinol (ALLO), as well as erythropoietin (Epo), human amnion epithelial cells (hAEC) and melatonin, which are showing promise in preclinical studies. We will discuss their use as treatments, either pre- or postnatally, for ventilation-induced brain injury and their known effects on the pathways involved in ventilation-induced injury, depicted in figure 1. The articles relevant to the purpose of this review are summarized in table 1.

Prenatal Therapies

Maternal Glucocorticoid Therapy

The synthetic glucocorticoids betamethasone and dexamethasone are routinely administered to women at risk for preterm delivery to accelerate maturation of the fetal lungs, so preterm infants can better transition at birth. The introduction of antenatal corticosteroid treatment has reduced respiratory distress syndrome rates by 34% and neonatal death rates by 31% [19], making it the cornerstone of modern perinatal care.

Studies in lambs demonstrated that betamethasone or dexamethasone treatment prior to high V_T ventilation is

Table 1. Therapeutic effects of maternal glucocorticoids, ALLO, Epo, hAEC and melatonin on the preterm brain

	Stabilizes haemodynamics	Anti-inflammatory	Anti-apoptotic	Anti-oxidative stress	Decreases the permeability of the blood-brain barrier
Maternal glucocorticoid therapy	√	Unclear	Unknown	Unknown	Unknown
Melatonin	Can have vasoconstrictive and vasodilatory effects depending on the dose	√	√	√	√
ALLO	Unknown	Unknown	Unknown	√	Unknown
Epo	Unknown	√	√	√	√
hAEC	Unknown	√	Unknown	Unknown	√

The key causes of neonatal brain injury, all of which are involved in ventilation-induced brain injury, and whether maternal steroid therapy, ALLO, Epo, hAEC and melatonin are able to attenuate these pathways, are shown. This gives an indication of which therapeutics have the potential to prevent brain injury associated with injurious ventilation in the delivery room.

sufficient to protect against ventilator-induced lung injury; lambs had improved ventilation parameters as well as reduced lung inflammation and gross injury [20], consistent with a lower peak inflation pressure and FiO_2 and a shorter duration of ventilation in human preterm neonates [21]. The effects of steroid treatment on the developing brain are less well understood, particularly after short-term ventilation. However, betamethasone (administered 24 h prior to birth) and dexamethasone (administered after delivery prior to ventilation onset) did not reduce ventilation-induced systemic inflammation, suggesting that steroids are unable to prevent the systemic spread of the pulmonary inflammatory response [20]. Despite this, the ability of steroids to improve ventilation parameters may assist in maintaining haemodynamic stability. Studies in sheep have shown that betamethasone treatment decreases fetal pulmonary vascular resistance and subsequently increases pulmonary blood flow, leading to an improved cardiopulmonary transition at birth in preterm lambs [22]. Other studies have found significant vasoconstriction in the near-term brain despite hypercapnia, potentially protecting against fluctuations in the cerebral blood flow [23]. Collectively, the improved transition at birth coupled with cerebral vasoconstriction may be the underlying link between maternal steroid therapy and the well-established reduced risk of IVH in preterm infants [24]. Further studies investigating the interaction between antenatal glucocorticoid treatment and postnatal interventions on the immature brain are still required.

Melatonin

Melatonin is an endogenous neurohormone that plays an essential role in providing circadian and seasonal timing cues, with high levels of synthesis at night. Melatonin has potential neuroprotective actions, which may be mediated by a number of physiological actions. Melatonin's neuroprotective capabilities are thought primarily to arise from its high effectiveness as an antioxidant both as a direct scavenger of oxygen free radicals, particularly the highly destructive hydroxyl radical [25, 26], and as an indirect antioxidant via stimulation of antioxidant enzymes [25, 26]. Melatonin also demonstrates anti-inflammatory properties, reducing upregulation of pro-inflammatory cytokines by preventing the translocation of nuclear factor (NF)- κ B [25]. Melatonin also has vasoactive properties that may provide benefit in the perinatal period under conditions of compromise [27, 28]. For the fetal or neonatal brain, melatonin has a number of additional important properties. It readily crosses the placenta and the blood-brain barrier and it does not have any apparent adverse effect for the mother or baby, even in high concentrations [29–31].

In light of the inflammatory and pro-oxidative stress environment that results from ventilator-induced lung injury, melatonin appears to be a compound with multiple attributes to protect the brain. In preterm infants on mechanical ventilation and with respiratory distress syndrome, postnatal treatment with melatonin reduces lung aspirate and serum concentrations of pro-inflammatory cytokines and markers of oxidative stress [32, 33]. Melatonin administration to pregnant sheep mitigates the brain production of hydroxyl radical and cellular lipid

Fig. 1. Central pathways involved in ventilation-induced lung and brain injury. The pathways through which glucocorticoids, ALLO, Epo, hAEC and melatonin are effective are represented by the respective coloured circles. Glucocorticoids, hAEC and melatonin have the potential to prevent the amplification of injury in the lungs and the passage to the brain, whereas ALLO and Epo are more brain specific in their effects.

peroxidation in response to acute fetal hypoxia [31]. Antenatal melatonin decreases white-matter brain injury characterized by hypomyelination and axonal damage and improves motor and cognitive function in newborn lambs after experimental intrauterine growth restriction in sheep [29].

The effect of melatonin on ventilation-induced brain injury has not been investigated. However, prenatal melatonin administration does reduce heart and coronary vessel dysfunction in lambs exposed to chronic feto-placental hypoxia and intrauterine growth restriction [27], and postnatal melatonin protects cerebrovascular function in chronically hypoxic newborn lambs to maintain cerebral perfusion [28], suggesting that melatonin may be effective at stabilizing haemodynamics. Further, melatonin decreases brain inflammation and protects the structural integrity of the blood-brain barrier in response to acute hypoxia in fetal sheep [34]. Thus, melatonin may target both haemodynamic instability and inflammation pathways of ventilation-induced brain injury, making it an attractive adjunct therapy for future research.

Postnatal Therapies

Allopurinol

ALLO can directly, and indirectly, protect the neonatal brain from hypoxia-ischaemia. Hypoxia-ischaemia leads to hypoxanthine build-up in cells, which is metabolized to superoxide and hydroxyl radicals by xanthine oxidase; ALLO functions by inhibiting xanthine oxidase and directly scavenging free radicals [35]. Further, ALLO can convert to oxypurinol, which is capable of crossing the blood-brain barrier and also inhibiting xanthine oxidase [36].

The effects of ALLO have been investigated in term infants with hypoxic-ischemic encephalopathy. ALLO reduced S100 β and neuroketal, both biomarkers of brain injury [37]. Term infants who received ALLO after severe asphyxia had decreased free radical formation, improved electrical brain activity and decreased mortality [38], without longer-term increased morbidity [39]. However, a multicentre randomized placebo-controlled trial showed that maternal treatment with ALLO during fetal

hypoxia did not reduce cord blood biomarkers of neuronal damage [40]. Further, ALLO treatment of a cohort of preterm infants with periventricular leucomalacia and retinopathy of prematurity, and elevated levels of oxidative markers, showed no clinical benefit [41].

To date, ALLO treatment at the initiation of ventilation has not been investigated. ALLO has not been effective at reducing chronic lung disease and BPD in preterm infants [42] and, since recent trials have not demonstrated a profound neuroprotective effect of ALLO in preterm infants, it might best be investigated as an adjunct therapy with improved respiratory care. There is also no clear difference between pre- and postnatal treatment, so timing of treatment also requires elucidation. Given that an ideal adjunct therapy for ventilation-induced brain injury should be administered soon after ventilation onset, we recommend investigating ALLO as an early postnatal treatment. We believe that, given that oxidative stress is a critical pathway through which ventilation can harm the lungs and brain, ALLO may provide acute protection against the activation of oxidative stress pathways.

Erythropoietin

Epo is a glycoprotein cytokine involved in erythropoiesis. It has antiapoptotic, anti-inflammatory and neuroregenerative properties and is used for treatment of adult stroke and anaemia of prematurity [43].

The efficacy of Epo as a neuroprotective agent has been extensively studied in term animal models. In rat and sheep models of perinatal brain injury, high-dose human recombinant Epo was not only safe and well tolerated but also reduced cell death, microgliosis, astrogliosis and blood-brain barrier leakage [44–46]. These findings led to the investigation and demonstration of Epo as a neuroprotective therapy in term infants with hypoxic-ischemic encephalopathy, with no adverse effects noted [47]. Lower doses of Epo administered over a longer duration from within the first 48 h of life to 2 weeks after birth reduced disability, and the incidence of cerebral palsy and death, in term infants with moderate hypoxic-ischemic encephalopathy [48].

Less is known about the effects of Epo in preterm infants. One clinical trial of low-dose Epo 3 times per week found a trend for improved neurological outcomes and a reduced requirement for blood transfusions [49]. In a school-age follow-up study, 9% of preterm infants who received high-dose Epo for up to weeks after birth demonstrated major neurological impairments compared to 23% of their control group. Furthermore, they found normal development and a regular school attendance in only

6% of their control group compared to 52% of the Epo-treated group, all of whom had IVH in the early newborn period [50]. In contrast, in 142 preterm infants (<1,250 g at birth) to whom Epo (400 IU/kg) was administered 3 times per week from day 4 of life for 8–10 weeks, there were no differences in most indices assessed. There was, however, an increased incidence of severe IVH in the Epo group (12.2 vs. 2%) and a significantly greater number with an abnormal psychomotor developmental index (31 vs. 13%; $p < 0.05$) assessed at 18–22 weeks' corrected age [51]. Hence, based on current data, the efficacy of Epo for preterm infants is not clear; clinical trials are continuing to clarify the outcomes of its use.

The majority of studies using Epo delay the onset of treatment. Relatively few studies have assessed the effect of Epo given hours, rather than days, after birth. One clinical trial in preterm infants with multiple high doses of Epo given as early as 3 h after birth found trends of increased IVH, periventricular echodensity and neonatal death [52]. In contrast, a more recent trial using the same dosing regimen found a reduction in white and grey matter loss using magnetic resonance imaging in infants treated with Epo [53]. Animal studies are now investigating whether Epo maintains its neuroprotective capacity when administered soon after birth. When a single high dose of Epo was administered to lambs receiving high V_T ventilation, lung inflammation and injury were amplified compared to results with a high V_T alone [54]. Within the brain, Epo treatment had regional effects; gene expression of pro-inflammatory cytokines was elevated in the periventricular white matter after Epo administration, but there was a reduction in the size and density of microglial aggregations, decreased astrogliosis and increased integrity of the blood-brain barrier in the subcortical white matter [14]. These findings suggest a potential toxicity of early Epo treatment in preterm ventilated newborns. We believe that it is not a good candidate for therapy at this time but certainly warrants further investigation.

Human Amnion Epithelial Cells

Cell therapies offer great promise for the prevention and treatment of tissue injury in newborns [55, 56]. The placenta offers a rich source of therapeutic cell types, and the amnion, in particular, offers exciting possibilities for perinatal therapy. The amnion, i.e. the membrane that surrounds the developing fetus, has been used clinically for wound healing for decades [57, 58]. Epithelial cells can be abundantly isolated from the amnion and demonstrate reparative and regenerative properties.

Briefly, the derivation of the amnion from the epiblast occurs prior to gastrulation, resulting in amnion epithelial cells having a pluripotent capacity similar to that of embryonic stem cells [59, 60]. This has been extensively characterized; epithelial cells derived from the amnions of term human pregnancies express molecular markers of pluripotent stem cells and can be differentiated in vitro down ectodermal, mesodermal, and endodermal lineages [59]. These findings render hAEC a realistic candidate for use as a regenerative therapeutic agent [61]. Their collection during a caesarean section from tissue otherwise discarded means they do not raise the same ethical concerns as stem cells derived from human embryos. In addition, several other attributes of hAEC make them attractive for use as a cell-based therapy: they have low immunogenicity, maintain their karyotype in culture, have long telomere lengths and a high telomerase activity and, unlike embryonic stem cells, do not form teratomas in vivo [59, 61, 62].

hAEC have the capacity to protect and repair the neonatal lung. In fetal sheep ventilated in utero for 12 h, hAEC administration reduced collagen and elastin deposition and decreased fibrosis when lungs were analysed 7 days later; hAEC had also differentiated into type I and II alveolar cells [63]. hAEC also protected the fetal lung against inflammation-induced damage; hAEC delivered in utero 0, 6 and 12 h after intra-amniotic LPS improved the tissue-to-airspace ratio and the septal crest density, reduced inflammatory cytokines, and increased surfactant protein mRNA levels in the lungs 7 days after treatment [64]. Given the success of these experimental studies, a trial has commenced investigating the safety of intravenously administered hAEC for preterm infants at <28 weeks of gestation with established bronchopulmonary dysplasia at a postmenstrual age of 36 weeks (trial ID ACTRN12614000174684).

The effects of hAEC on neonatal brain injury are less explored. hAEC are able to differentiate along the ectodermal lineage and can differentiate into neuronal and glial cells [65]. They express neural genes and secrete multiple neurotransmitters [66, 67]. Only one study to

date has demonstrated neuroprotective and anti-inflammatory responses after intravenous and intratracheal hAEC administration in the neonatal brain following inflammation-induced brain injury in fetal sheep [68]. Given that hAEC are able to protect the preterm lung from in utero ventilation- and inflammation-induced lung injury [63, 64], and that they protect the brain from in utero inflammation-induced damage [68], they may also have the potential to protect against ventilation-induced brain injury as a postnatal treatment. We observed an apparent beneficial effect of hAEC on brain inflammation as a result of high V_T ventilation; hAEC (administered both intravenously and intratracheally) reduced microgliosis and vascular protein extravasation in the brain 2 h after of ventilation onset [9]. However, hAEC did not stabilize the haemodynamic transition following ventilation onset, yet this pathway deserves further investigation. These initial observations suggest that cell therapies alone may not be sufficient to prevent ventilation-induced brain injury, but their anti-inflammatory and reparative characteristics may make them an effective adjunct treatment.

Conclusion

Many preterm infants will require some form of respiratory support in the delivery room, often in the form of IPPV. Both manual and mechanical ventilation in the delivery room is not well controlled and can be inadvertently injurious to the preterm lungs and brain. Even minimally invasive ventilation strategies can result in brain injury in preterm infants, so there is a critical need for therapies. The ultimate therapy would have the capacity to stabilize cardiopulmonary-cerebral haemodynamics and reduce lung, systemic and brain inflammation in order to reduce ventilation-induced lung and brain injury. We hope that with additional research the optimal therapy to minimize the burden of ventilation-induced brain injury on preterm infants will be identified.

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