Initiation of Resuscitation with High Tidal Volumes Causes Cerebral Hemodynamic Disturbance, Brain Inflammation and Injury in Preterm Lambs

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Abstract

Aims: Preterm infants can be inadvertently exposed to high tidal volumes (VT) in the delivery room, causing lung inflammation and injury, but little is known about their effects on the brain. The aim of this study was to compare an initial 15 min of high VT resuscitation strategy to a less injurious resuscitation strategy on cerebral haemodynamics, inflammation and injury.

Methods: Preterm lambs at 126 d gestation were surgically instrumented prior to receiving resuscitation with either: 1) High VT targeting 10–12 mL/kg for the first 15 min (n = 6) or 2) a protective resuscitation strategy (Prot VT), consisting of prophylactic surfactant, a 20 s sustained inflation and a lower initial VT (7 mL/kg; n = 6). Both groups were subsequently ventilated with a VT 7 mL/kg. Blood gases, arterial pressures and carotid blood flows were recorded. Cerebral blood volume and oxygenation were assessed using near infrared spectroscopy. The brain was collected for biochemical and histologic assessment of inflammation, injury, vascular extravasation, hemorrhage and oxidative injury. Unventilated controls (UVC; n = 6) were used for comparison.

Results: High VT lambs had worse oxygenation and required greater ventilatory support than Prot VT lambs. High VT resulted in cerebral haemodynamic instability during the initial 15 min, adverse cerebral tissue oxygenation index and cerebral vasoparalysis. While both resuscitation strategies increased lung and brain inflammation and oxidative stress, High VT resuscitation significantly amplified the effect (p = 0.014 and p < 0.001). Vascular extravasation was evident in the brains of 60% of High VT lambs, but not in UVC or Prot VT lambs.

Conclusion: High VT resulted in greater cerebral haemodynamic instability, increased brain inflammation, oxidative stress and vascular extravasation than a Prot VT strategy. The initiation of resuscitation targeting Prot VT may reduce the severity of brain injury in preterm neonates.

Introduction

Brain injury, particularly white matter injury and intracranial hemorrhage, is a major problem in very premature infants and the incidence and severity increases with decreasing gestational age and birth weight [1]. In addition to immaturity of the brain at the time of preterm birth, these babies may be exposed to systemic pathogenic factors such as infection/inflammation (both ante- and post-natal) and disturbed cerebral blood flow [2,3,4]. In the immediate newborn period, resuscitation, mechanical ventilation, poor ventricular function, rapid volume expansion and a patent ductus arteriosus are strongly associated with cerebral pathology in immature newborns [2], suggesting that early respiratory and cardiovascular care of preterm infants may critically influence the immature brain and the progression towards brain injury.

Recent clinical trials find that many preterm infants are inadvertently exposed to high tidal volumes (VT) during the initial resuscitation in the delivery room [5,6]. The initiation of resuscitation is usually provided manually and the delivered VT is poorly controlled and not routinely measured [7]. However, the
initiation of respiratory support after birth, particularly with high VT, can induce an inflammatory response leading to chronic diseases of the lung such as bronchopulmonary dysplasia [8,9].

We, and others, have shown that resuscitation with high VT for 15 min initiates a pulmonary inflammatory response [10,11,12], which increases systemic proinflammatory cytokine mRNA expression [13,14,15]. Further, the initiation of ventilation with high airway pressures destabilizes the cardiopulmonary transition by increasing pulmonary vascular resistance, decreasing pulmonary blood flow, re-establishing right-to-left shunting through the ductus arteriosus and ultimately reducing left ventricular output [16]. These cardiovascular disturbances may underlie the pathogenesis of brain injury [17], acting via a reduction in cerebral blood flow indicated by reduced superior vena cava flow. Low SVC flow in the first 24 h after birth in preterm infants (<28 weeks), is strongly associated with IVH and long-term neurodevelopmental disability [18,19]. We have recently shown that cerebral haemodynamics were most affected by resuscitation within the first 15 min [20], coinciding with the major events of the cardiopulmonary transition at birth [21].

Given that the initial resuscitation of the preterm neonate, particularly with the use of high VT, can result in a pulmonary and systemic inflammatory response and cause cardiopulmonary haemodynamic instability and hypoxia, we aimed to investigate the effects of resuscitation on the preterm brain. Specifically, we compared cerebral haemodynamic and brain histology using two different resuscitation strategies, one using high VT and therefore considered injurious, versus a protective package of care, consisting of prophylactic surfactant, a sustained inflation followed by low VT resuscitation, designed to optimize transition with minimal barotrauma.

Materials and Methods

Ethics Statement

The experimental protocol was performed in accordance with guidelines established by the National Health and Medical Research Council of Australia and was approved by the Monash Medical Centre (MMCA) animal ethics committee at Monash University.

Instrumentation and Delivery

At 126±2 d (mean ± SD), the ewe and fetus were anesthetized (2% Isoflurane in oxygen, Bomac Animal Health, NSW, Australia) and the fetal head and neck were exposed via cesarean section. A skin incision was made in the fetal neck for placement of ultrasonic flow transducers (3 mm; Transonic Systems, Ithaca, NY) around the left and right carotid arteries. Carotid blood flow correlates highly with cerebral blood flow [22]. A polyvinyl catheter was placed into the left jugular vein. The fetal trachea was intubated with auffed endotracheal tube (4.0 mm) and lung liquid was drained passively. A transcutaneous oximeter (Masimo, Irvine, CA) was placed over the fronto-parietal region and covered with light-proof dressing, as described previously [28]. Polyvinyl catheters were placed into an umbilical artery to measure mean arterial pressure. All lambs received sedation (Alfaxan i.v. 5–15 mg/kg/h; Jurox, East Tamaki, Auckland, New Zealand) in 5% dextrose to minimize spontaneous breathing during the experiment via the umbilical vein catheter. Left and right carotid arterial blood flows (CABF) were recorded continuously (Powerlab; ADInstruments, Castle Hill, NSW, Australia), along with mean arterial pressure (DTX Plus Transducer; Becton Dickinson, Singapore), from before delivery until the end of the experiment. Unventilated controls (UVC; n = 6) at the same age were delivered via cesarean section and immediately killed (sodium pentobarbital: >100 mg/kg i.v.) without undergoing surgery or postnatal anaesthesia. Ewes were similarly humanely killed after delivery of the lambs.

Resuscitation Strategy

Lambs were randomly assigned to receive either of two strategies that, based on previous studies, would be expected to either cause lung injury (High VT group; n = 6) or to protect the lung from injury (Prot VT group; n = 6). These were: 1) High VT, targeting a volume of 12–15 mL/kg within the first postnatal breaths using a neonatal positive pressure ventilator (Babylog 9000+, Dräger, Lubeck, Germany), which was maintained for 15 minutes to mirror the time taken for clinical neonatal resuscitation and transfer to NICU [23]; or 2) Prot VT, consisting of prophylactic surfactant (100 mg/kg, Curosurf®, Chiesi Pharma, Italy) followed by a 20 second sustained inflation delivered by a Neopuff (Fisher & Paykel Healthcare, Panmure, Auckland, New Zealand) using a peak inspiratory pressure (PIP) of 35 cmH2O. This second package of care was chosen to minimize pulmonary and systemic inflammation and improve the cardiopulmonary circulatory transition at birth [24,25,26,27]. After the sustained inflation in the Prot VT group and after 15 min of ventilation in High VT group, all lambs were ventilated in volume guarantee mode (VT 7 mL/kg; PEEP, 5 cmH2O; inspiratory time, 0.3 s; and expiratory time, 0.6 s) for the remainder of the experiment. The high VT group did not receive surfactant. The total ventilation time for both groups was 90 minutes. In all lambs, ventilation was conducted using warmed, humidified air with the fraction of inspired oxygen initially set at 0.4 but adjusted to maintain arterial oxygen saturation (SaO2) between 88–95%. Well-being of the newborn lamb was monitored by regular blood gas analysis (ABL30, Radiometer, Copenhagen, Denmark) and pre-ductal transcutaneous oxyhemoglobin saturation.

Left ventricular output and the proportion of left-to-right to right-to-left shunting through the ductus arteriosus was detected using pulsed Doppler ultrasound as described previously [16]. The ductal shunt was calculated as a DA ratio (time left to right/right total shunt time). Spatially resolved spectroscopy (SRS, NIRO 200 Spectrophotometer; Hamamatsu Photonics K.K., Hamamatsu City, Japan) was employed for continuous recording of cerebral oxygenation expressed as a tissue oxygen index (TOI, %) at 6 Hz. The NIRO 200 also provides continuous measurements of changes in concentrations (µMolar.cm) of oxyhemoglobin (HbO2), deoxyhemoglobin (HHb) and total haemoglobin (HbT = HbO2+HHb). Two aligned photodetectors are housed in the detection probe which is spaced 4 cm from the emission probe, with both the emission and detection probes placed over the fronto-parietal region and covered with light-proof dressing, as described previously [28]. Changes in Cerebral Blood Volume (ΔCBV, ml/100 g) were calculated from measurements of the ΔHbT, using a differential path-length factor of 4.99 [28,29] and formula as shown below [30].

Calculations

Dynamic compliance (Cdyn), adjusted for weight, was calculated as $V_L/\text{kg}/(\text{PIP} - \text{PEEP})$. Ventilatory efficiency index (VEI) was determined as $3800/(\Delta P - f_\text{pCO}_2)$ where 3800 is a constant for the production of carbon dioxide (ml·min·kg$^{-1}$·min$^{-1}$), $\Delta P = \text{PEEP} - \text{PEEP}$ and $f$ is the respiratory frequency [31]. Arterial oxygenation was described using the alveolar-arterial difference in oxygen (AaDO2) [32]. Oxygenation Index was calculated as $\text{OI} = (\text{FiO}_2 \cdot \text{mean airway pressure})/\text{PaO}_2 \cdot \Delta\text{CBV} = (\Delta\text{HBT} \cdot \text{FiO}_2 \cdot \text{mean airway pressure})/\text{PaO}_2$.
$MW_{\text{Hemoglobin}} = 10^{-6}/(tHb \cdot 10^{-2} \cdot \text{CLVHR} \cdot \text{Dt} \cdot 10)$ where $MW_{\text{Hemoglobin}}$ = molecular weight of hemoglobin = 64,500, $tHb$ = concentration of hemoglobin in g/100 mL$^{-1}$, CLVHR = cerebral to large vessel hematocrit ratio $= 0.69$, and DT = brain tissue density in g/mL$^{-1} = 1.05$.

Lung and Brain Collection and Processing

At the conclusion of the ventilatory protocol, lambs were humanely killed, weighed and the lungs and brains collected. All histological and molecular assessments of resuscitated lambs were compared to UVC to determine the comparative effects of different resuscitation strategies on lung and brain inflammation and injury. The lung was removed and sections of the right lower lobe were snap-frozen in liquid nitrogen for subsequent measurement of interleukin [IL-1β, IL-6 and IL-8 mRNA expression using quantitative RT-PCR [33]. The brain was removed and sectioned as described previously [34]. The left hemisphere was sectioned into approximately 4-mm slices, and fixed in 4% paraformaldehyde for histological and immunohistochemical analyses. Serial sections (10 μm) at the level of the lateral ventricle were stained with: hematoxylin and eosin (H&E) for gross assessment of brain pathology. Immunohistochemistry was used to assess: the presence of inflammatory cells using peroxidase-labeled lectin (1:200, Sigma, USA); lipid peroxidation (4-Hydroxynonenal; 4HNE, 1:1000, Calbiochem, USA); and blood brain barrier integrity (serum albumin, 1:1000, Accurate Chemical & Scientific Corporation, USA). Lectin- and 4HNE-positive cells were counted in four random non-overlapping high-powered fields in subcortical and periventricular white matter of the lateral ventricle were stained with: hemotoxylin and eosin (DP2-BSW, Olympus, Japan). For H&E sections, each field was designated a qualitative score for the assessment of brain injury, where 0 = none, 1 = mild (<50% of the field of view occupied by brain damage), 2 = severe (>50% of the field of view occupied by brain damage). Albumin extravasation was determined by the presence or absence of albumin within the tissue surrounding blood vessels; only the incidence was noted.

Statistical Analysis

Data are presented as mean ± SEM. Serial data were compared between groups using two-way ANOVA with repeated measures (Sigmastat v3.0, SPSS Inc.). The first 15 minutes was analysed separated as above. Posthoc comparisons were performed using the Holm-Sidak method. ANOVA was used to compare biochemical and histologic indices of injury. Statistical significance was accepted for $p<0.05$.

Results

Umbilical arterial blood gas and acid/base status at birth were normal for all lambs and not different between groups (Mean for all groups: pH: 7.19±0.05 PaCO₂: 67.0±5.8 mmHg; PaO₂: 28.8±2.5.1 mmHg; SaO₂: 60.3±8.7%). Lamb body weights were not different between groups (UVC: 2.9±0.2 kg; Prot V₁: 3.2±0.2 kg; High V₁: 3.1±0.2 kg; $p = 0.600$).

Ventilation and Oxygenation

Peak inspiratory pressure was higher in the High V₁ group during the initial 15 min resuscitation period, as expected, and remained higher for the subsequent 75 min ventilation period ($p = 0.002$; Figure 1A) compared to the Prot V₁ group. The mean tidal volume obtained in the High V₁ group in the first 15 minutes was 12.5±2.3 mL/kg (Range 10.9–13.4 mL/kg) while in the Prot V₁ group it was 7.0±0.2 (Range 6.7–7.2 mL/kg; $p<0.001$; Figure 1B). V₁ was not different after the initial 15 minutes. Lung static compliance was lower (worse) in High V₁ lambs from 30 min ($p<0.001$; Figure 1C), while required FiO₂ was higher in High V₁ lambs throughout the ventilation procedure ($p = 0.028$; Figure 1D). Calculated airway resistance was significantly higher in High V₁ lambs compared to Prot V₁ group lambs (High V₁: 82.1±4.9, Prot V₁: 48.7±5.4; $p = 0.002$).

The partial pressure of arterial oxygen ($PaO₂$) was higher at 10 and 15 min in High V₁ lambs but variability precluded significance ($p = 0.266$; Figure 2A). $PaCO₂$ was significantly lower in High V₁ lambs during the 15 min resuscitation period ($p<0.001$; Figure 2B), but was not different thereafter. Oxygenation Index was higher ($p = 0.003$; Figure 2C) and the alveolar-arterial difference in oxygen was lower ($p = 0.039$; Figure 2D) throughout resuscitation and ventilation in High V₁ lambs, indicative of poorer oxygenation. Arterial oxygen saturation was successfully maintained in both groups (High V₁: 87.1±2.8%, Prot V₁: 88.1±2.9%) and was not different between groups ($p = 0.393$). Calculated cerebral oxygen delivery was not different between groups at any of the blood-gas time points ($p = 0.840$). Body temperature ($p = 0.784$), blood lactate ($p = 0.893$) or blood glucose ($p = 0.435$) were not different between groups (data not shown).

Haemodynamics

Combined left and right CaBF were significantly higher within the first 10 min in High V₁ lambs compared with Prot V₁ lambs (Fig 3 A & B). The coefficient of variation in total CaBF was significantly higher in High V₁ lambs compared to Prot V₁ (mean: High V₁: 60.6±0.06 vs. Prot V₁: 38.2±0.05; $p<0.01$); the majority of the variability was observed within the first 15 min. Maximum CaBF ($p<0.001$) and the amplitude of CaBF ($p = 0.002$) was significantly higher within the first 15 min in High V₁ lambs compared to Prot V₁. Mean arterial pressure was significantly higher in High V₁ lambs between 10–15 min and at 60 min compared to Prot V₁ lambs (Fig 3C). Left ventricular output ($p = 0.57$), heart rate ($p = 0.14$) and DA Ratio ($p = 0.28$) were not different between groups. LVO and heart rate decreased similarly in both groups over time ($p<0.001$).

SRS-NIRS

NIRS data was successfully obtained from 4 Prot V₁ and 6 High V₁ lambs. Averaged Tissue Oxygenation Index (Figure 4A & B) was not different between groups, but TOI variations between and within individual lambs were much greater in the High V₁ group. Changes in cerebral blood volume from baseline measurements was significantly lower in High V₁ lambs compared to Prot V₁ lambs from 60 min ($p<0.001$; Figure 4C & D). The Prot V₁ group showed a significant fall in CBV from baseline ($p<0.001$), while the High V₁ group remained unchanged.

Inflammation and Oxidative Injury

Lung mRNA cytokine expression of IL-1β, IL-6 and IL-8 was significantly elevated in High V₁ lambs compared to Prot V₁ lambs and UVC (Fig 5). Within the subcortical white matter of the brain, the number of lectin-positive cells was significantly higher in the High V₁ group compared to UVC ($p = 0.014$; Table 1). The Prot V₁ group was not different to UVC ($p = 0.072$). There were no differences observed in the periventricular white matter...
The number of 4HNE-positive cells, indicative of oxidative injury, was significantly higher in High VT lambs within the periventricular white matter compared to UVC (p < 0.001) and showed a strong trend to be greater than Prot VT lambs (p = 0.054). There was no difference observed in the subcortical white matter (p = 0.13; Table 1).

**Figure 1.** Peak inspiratory pressure (A), tidal volume (B), dynamic compliance (C) and the fraction of inspired oxygen (FiO₂; D) in High VT (open circles) and Prot VT (closed circles) lambs. *p < 0.05 High VT vs. Prot VT.

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**Figure 2.** The partial pressure of arterial (Pa) carbon dioxide (CO₂; A), oxygen (O₂; B), oxygenation index (C) and the alveolar-arterial difference in oxygen (AaDO₂; D) in High VT (open circles) and Prot VT (closed circles) lambs. *p < 0.05 High VT vs. Prot VT.

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Figure 3. Left (A) and right (B) carotid blood flow (CABF) and mean arterial pressure (C) in High VT (open circles) and Prot VT (closed circles) lambs. *p<0.05 High VT vs. Prot VT. doi:10.1371/journal.pone.0039535.g003

Figure 4. Tissue oxygenation index (TOI; A & B) and cerebral blood volume (C & D) in High VT (open circles) and Prot VT (closed circles) lambs. Values are derived using spatially resolved near infrared spectroscopy. Note the greater variability in TOI within the first 15 min during High VT lambs (B) compared to Prot VT. Cerebral vascular vasoparalysis is evident in 4 of 5 High VT lambs by the maintenance of near stable cerebral blood volume (D). doi:10.1371/journal.pone.0039535.g004
Brain Pathology and Hemorrhage

The incidence and severity of gross anatomical brain injury within the subcortical and periventricular white matter was higher in resuscitation groups compared to UVC, but no difference was observed between resuscitation groups receiving High VT compared to Prot VT (Table 1). The incidence of vascular extravasation, as evidenced by protein leakage, was evident in 60% of High VT lambs, but was not observed in Prot VT or UVC (p = 0.030; Table 1).

Discussion

Our previous studies suggest that the initial 15 minutes of resuscitation after birth may be a critical time period for the development of brain inflammation and injury in preterm infants [20]. The findings of our current study are consistent with this contention and further demonstrate that the ventilation strategy used in this critical time period after birth can adversely affect the developing brain. Importantly, the use of high tidal volumes for resuscitation alters cerebral haemodynamics, increases brain inflammation, oxidative stress and vascular extravasation compared to a Prot VT protective resuscitation protocol.

Although preterm infants can be inadvertently exposed to high VT during the initial resuscitation in the delivery room [5,6] that can result in lung inflammation and injury [8,9,11,14], the consequences for the brain have not been investigated. The principal pathogenic factors causing neonatal preterm brain injury are infection/inflammation (both ante- and post-natal) and disturbed cerebral blood flow [2,3,4]. These factors can occur from aggressive resuscitation of preterm neonates [14,20], which can also adversely affect and destabilize cardiac function leading to a much greater risk of cerebral vascular injury.

In the Prot VT group we aimed to achieve stable arterial blood gas levels and to avoid large fluctuations in intra-thoracic pressure that disturb cardiac function. We subsequently showed that cerebral oxygenation was also relatively stable and that CBV gradually reduced in all Prot VT lambs. The normal postnatal reduction in cerebral blood volume and cerebral blood flow is a well-established and described phenomenon in both animals [35] and humans at term [36,37] and preterm [38]. It results from the normal postnatal increase in oxygen content and demonstrates the importance of oxygen delivery in regulating CBF [39]. In contrast, lambs subjected to High VT resuscitation failed to display a decrease in CBV, despite similar or higher (first 15 min) blood oxygenation levels, suggesting that the normal postnatal adaptation of the cerebral circulation has been altered in these lambs. Furthermore, as these lambs also had lower PaCO2 levels during the 1st 15 min, we would expect this to enhance the cerebrovasoconstriction caused by increased oxygenation, providing further evidence that the cerebral vascular response was abnormal in High VT lambs. It is possible that this reflects injury to the cerebral vessels and likely contributed to the vascular leakage observed in the High VT group, particularly as relative cerebral hyperemia and cerebral vasoparalysis are known features in neonatal brain injury [40,41]. Our data on the carotid blood flow variations also

Table 1. Assessment of brain injury.

| Group                              | UVC (n = 6) | Prot VT (n = 5) | High VT (n = 5) |
|------------------------------------|-------------|----------------|----------------|
| Incidence and severity of injury (%) |             |                |                |
| **Gross Anatomical Injury**        |             |                |                |
| Periventricular white matter       | 0           | 0              | 0              |
| Subcortical white matter           | 12          | 0              | 12             |
| Lipid Peroxidation                 |             |                |                |
| Periventricular white matter       | 245 ± 99    | 412 ± 41       | 580 ± 60*      |
| Subcortical white matter           | 271 ± 73    | 393 ± 38       | 402 ± 39       |
| Vascular protein extravasation     |             |                |                |
| Periventricular & subcortical white matter | 120 ± 37 | 205 ± 26 | 211 ± 65 |
| Infiltrating inflammatory cells    |             |                |                |
| Periventricular white matter       | 196 ± 58    | 536 ± 40       | 728 ± 242*     |
| Subcortical white matter           |             |                |                |

Incidence and severity of gross anatomical brain injury expressed as % of the lambs displaying evidence of mild or severe injury. *indicates significant difference between High VT and UVC. **indicates significant difference between High VT and Prot VT.

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support the findings of IVH in 21 of 23 infants in which fluctuations in cerebral blood flow velocity (measured by Doppler ultrasound) were detected [42]. Coupled with our previous study [20], our findings show that cerebral haemodynamics during the immediate newborn period is critically dependent upon pulmonary blood flow and LVO, which in turn, are critically dependent on resuscitation strategy.

Our study highlights the ability to detect some aspects of brain injury, including inflammation, oxidative stress and microscopic hemorrhage in brains within 90 minutes after the onset of resuscitation/ventilation at birth. This has not been previously reported, with most commentaries indicating that it takes 24 to 48 hours before brain pathologies can be detected. In the clinical setting, most cerebral ultrasounds are performed more than 24 hours after birth, with MRI studies conducted much later, and only if the baby can be moved. The study protocol allowed us to determine the effects of the initial resuscitation strategy, without the confounding influences of prolonged maintenance of respiratory and life support and other postnatal influences including nutrition and sepsis.

To assess how high VT resuscitation impacts the immature brain, we compared these lambs with lambs resuscitated with a strategy we considered to be less injurious (Prot VT) and to minimally interfere with the cardiovascular transition at birth. The Prot VT group received prophylactic surfactant, a 20 second sustained inflation followed by volume guarantee ventilation at 7 mL/kg, representing the normal spontaneous tidal volume of preterm lambs [15]. We have previously shown that an initial sustained inflation (20 s) can fully aerate the lung before the onset of tidal breathing [24], thereby facilitating a smooth pulmonary blood flow transition immediately after birth [24], decreases early markers of lung injury and inflammation [25] and improves CaBF stability compared to controls [26]. Further, prophylactic surfactant administration decreases markers of lung inflammation caused by high tidal volumes at birth [27] and markedly improves the uniformity of lung aeration [43]. While resuscitation in both groups increased indices of brain injury, inflammation and oxidative stress, the Prot VT group displayed normal postnatal adaptive changes within the cerebral circulation and had reduced brain inflammation compared to the more injurious group. This suggests that improved resuscitation procedures, including appropriate lung recruitment and minimizing lung injury, may reduce the risk and severity of brain injury.

This study confirms that resuscitation with high VT for 1.5 min initiates a pulmonary inflammatory response [10,12,44] which increases systemic proinflammatory cytokine mRNA expression [13,14,15]. One of the major up-stream mechanisms of brain injury, particularly pertaining to cerebral white matter injury of prematurity, is inflammation/infection, mediated by a systemic up-regulation of inflammatory cytokines [3]. While most of the research on the role of pro-inflammatory cytokines in white matter injury has focused on intrauterine inflammation or postnatal injury has focused on intrauterine inflammation or postnatal inflammation, oxidative stress and microscopic hemorrhage in brains within 90 minutes after the onset of resuscitation/ventilation at birth. This has not been previously reported, with most commentaries indicating that it takes 24 to 48 hours before brain pathologies can be detected. In the clinical setting, most cerebral ultrasounds are performed more than 24 hours after birth, with MRI studies conducted much later, and only if the baby can be moved. The study protocol allowed us to determine the effects of the initial resuscitation strategy, without the confounding influences of prolonged maintenance of respiratory and life support and other postnatal influences including nutrition and sepsis.

In summary, this study has shown that resuscitation increases brain inflammation and oxidative stress, but an initial high VT resuscitation strategy further exacerbated cerebral hemodynamic instability, brain inflammation and injury. This study highlights the critical role that the initial respiratory support has on the development of brain inflammation and injury, and the requirement for better monitoring of delivered tidal volumes to preterm infants in the delivery room. It also demonstrates that brain injury can be seen within 90 minutes of the onset of an injurious stimulus, and the severity of brain injury can be reduced by protective resuscitation strategies in the delivery room.

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Author Contributions

Conceived and designed the experiments: GP SM FW AG TM MK SH. Performed the experiments: GP SM AB FW JA AG TM MK SH. Analyzed the data: GP SM AB FW JA MT. Contributed reagents/materials/analysis tools: GP SM AB FW JA MT. Wrote the paper: GP SM AB FW JA AG TM MT MK SH.

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