

● PERSPECTIVE

Targeting inflammation to reduce brain injury in growth restricted newborns: a potential treatment?

Introduction: Intrauterine growth restriction (IUGR) is commonly caused by placental insufficiency, resulting in a chronic hypoxic environment and subsequent abnormal fetal development. The developing brain is particularly vulnerable to IUGR conditions. Multiple causal factors associated with brain injury in fetal growth restriction include the timing of placental insufficiency, onset and subsequent severity of fetal compromise, fetal cerebrovascular response and the redistribution of brain blood flow. Although a significant proportion of IUGR infants exhibit adverse long-term neurological outcomes, relatively few studies have focused on the mechanisms of brain injury in the IUGR neonate. Clinical imaging studies of IUGR infants demonstrate alterations in grey matter and white matter volume and structure (Tolsa et al., 2004; Esteban et al., 2010; Padilla et al., 2015). Cortical grey matter volume is reduced by up to 28% compared with control infants (Tolsa et al., 2004) and both white and grey matter show structural changes (Esteban et al., 2010). These structural changes persist at 1 year of age and are associated with significant developmental disabilities (Tolsa et al., 2004; Esteban et al., 2010). Currently there are no interventions available that alleviate brain injury in the IUGR neonate. As the developing brain exhibits plasticity and the potential for regeneration following injury, determining the underlying mechanism of grey matter injury (neuronal loss) in the IUGR infant may provide evidence for the rational development of therapies for treatment of brain injury in these infants.

Key neurodevelopmental processes are likely to be disrupted in the IUGR infant's brain which may underlie adverse neurodevelopmental outcomes. Inflammation is associated with neuronal injury in acute neonatal hypoxic-ischemic (HI) animal models (Wixey et al., 2011a, b; Buller et al., 2012), and may be associated with neuronal injury observed during chronic hypoxic events such as IUGR. In humans, systemic inflammation has been reported to result in poorer neurological outcomes in IUGR neonates (McElrath et al., 2013). High concentrations of the proinflammatory cytokines tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6 and IL-8 in the blood of IUGR infants were found to correlate with increasing likelihood of abnormal neurodevelopment (McElrath et al., 2013). How systemic inflammation may mediate brain injury is the current focus of research in this area. Altered blood-brain barrier (BBB) permeability in the IUGR neonate may result in an infiltration of systemic inflammatory mediators into the brain. BBB breakdown may also facilitate brain-derived inflammatory cells entering the blood.

Inflammation in the neonatal brain:

Microglial cells: Microglial cells are involved in cellular pruning during normal development as well as in pathological conditions. Following trauma to the neonatal brain, microglia become activated, increase in number and migrate to sites of injury. A marked increase in activated microglial cells in both white matter and grey matter have been demonstrated after acute HI in the rodent preterm model (Wixey et al., 2011a, b, 2012). The sustained presence of activated microglia in the brain following HI has also been associated with ongoing white matter and neuronal damage. These findings are also evident in the human preterm HI affected neonate brain. The negative impact of activated microglia in the IUGR brain remains to be fully elucidated (Wixey et al., 2017) however a recent study in a rat model of chemically-induced IUGR, demonstrated severe inflammation in the brain as well as a delay in myelination (Pham et al., 2015). Neonatal rats with moderate growth restriction induced by prenatal hypoxia, have also demonstrated inflammatory microgliosis in the white matter. Activated microglia are largely responsible for the production of excessive levels of proinflammatory cytokines in the brain that are toxic to neurons and damaging to developing white matter.

Proinflammatory cytokines: Proinflammatory cytokines, such as IL-1 β and TNF- α , are small, cell signalling glycoproteins involved in communication between cells. Activated microglia are the major sources of these cytokines and regulate their production and actions. Increasing evidence suggests a link between the elevation of proinflammatory cytokines, severity of injury and poor neurodevelopmental outcomes in asphyxiated term neonates. In the preterm infant, the occurrence of cerebral palsy has been attributed, at least partially, to increased levels of proinflammatory cytokines in the brain. Examining the levels of proinflammatory cytokines in IUGR will uncover whether an inflammatory response in the IUGR neonatal brain is associated with neuronal injury and whether this increase is associated with adverse neurological outcomes. A recent study in a guinea pig model of IUGR demonstrated an increase in IL-1 β and TNF- α in the fetal brain which correlated with worsening brain injury (Guo et al., 2010). High concentrations of the proinflammatory cytokines have also been reported in the blood of IUGR infants (McElrath et al., 2013) however no human studies exist examining the potential effects in the IUGR brain of a systemic inflammatory response.

BBB disruption: The BBB is recognized as a complex structure whose development and maintenance relies upon the structural and functional support of the endothelial cells (EC) by tight junction molecules, the basement membrane, extracellular matrix proteins and several cell types including astrocytes, pericytes and microglia (collectively referred to as the neurovascular unit). Astrocytic end-feet ensheath the ECs and are thought to play a significant role in BBB function, although in early developing fetal brain it has been

suggested that they have more of a regulatory role. The ability of the BBB to regulate traffic and prevent entry of potentially noxious substances is critical to neuronal health and to safeguarding the integrity of neuronal network connectivity and brain activity. Many modulators from blood and brain can contribute to opening of the BBB, although under most circumstances this is transient – persistent alterations may be indicative of BBB dysfunction. Under neuropathological conditions the integrity of the BBB is compromised, mainly by astrocytes which release inflammatory cytokines that subsequently contribute to BBB dysfunction. Microglia are also capable of modulating BBB permeability, by secreting factors as well as inducing expression of chemokines and cell adhesion molecules. As a result of BBB compromise, inflammatory mediators and cells of the innate and adaptive immune system may access the central nervous system and further exacerbate inflammation and neuronal injury. In adult stroke, systemic inflammatory mediators are recruited to the BBB and can contribute to the progression and severity of brain injury. In the fetal sheep exposed to endotoxin, plasma albumin was found in several brain regions at 24 hours after exposure and lectin-positive cells observed around subventricular white matter blood vessels suggesting BBB permeability and the potential for entry of cytotoxic and proinflammatory. Understanding the contribution of both the innate and systemic inflammatory response will expose cellular targets that could be therapeutically exploited to improve neurological outcome in these infants.

Biomarkers in IUGR neonatal blood: Rapid determination of the extent of brain injury in the neonate is critical for early intervention. Few studies have examined blood biomarkers in the neonate to assess cerebral cell damage. Recent human studies have identified S100 β as a useful marker of brain injury in the IUGR neonate with significant increases in levels in asymmetric IUGR neonates, however there is some debate as to its robustness as a biomarker due to its presence in blood independent of injury. At one and two weeks of age, an increase in inflammatory markers (such as IL-1 β and TNF- α) in the blood of IUGR infants correlates with adverse behavioural outcome at 2 years of age, however more immediate identification for early intervention is critical. Defining inflammatory markers that correlate with early neuronal integrity and longer-term brain outcomes will be a major contribution to guide translation to clinical studies. Appropriate blood biomarkers could (i) be of direct use to clinicians in diagnosing the newborn at risk of brain injury quickly and accurately and (ii) aid in establishing whether therapeutic treatment recovers deficits.

Reducing inflammation to prevent brain injury: a potential treatment: As nearly three-quarters of newborns at risk of IUGR are not recognised until delivery it is important to examine the vulnerable IUGR neonatal brain to best determine treatment options to prevent long-term adverse neurological outcomes. Research to address IUGR health problems from both the preventative aspect *in utero* as well

as interventions from birth will maximise opportunities to improve neurodevelopmental outcomes for all IUGR babies.

Various postnatal neuroprotective therapies are being trialled in animal models of IUGR however only a handful of studies have examined effects on inflammatory mechanisms in the brain including inhaled nitric oxide (Pham et al., 2015) and erythropoietin (Mazur et al., 2010). Pham et al. (2015) demonstrated that 7 days of nitric oxide treatment during the first postnatal week to IUGR rats exposed to antenatal hypoxia significantly attenuated cell death and microglial activation and improved myelination. Erythropoietin is known to exert anti-inflammatory effects in the neonatal brain and reduce inflammatory cytokine production in term and preterm infants. In a rat model of placental insufficiency, Erythropoietin administration (2,000 IU/kg for 5 days from postnatal day 1) was found to enhance oligodendrocyte and neuronal survival in adult offspring, although neuroinflammatory markers were not directly examined.

Ibuprofen is commonly used in the neonate and has anti-inflammatory properties. Ibuprofen is routinely used for the treatment of patent ductus arteriosus in the preterm neonate and is safe and well tolerated. Although the potential neuroprotective effects of ibuprofen administration have not been examined in animal models of IUGR we can be guided by evidence from neonatal animal models of acute HI. In a neonatal rat HI model, post-insult administration of ibuprofen (100 mg/kg day 1 and 50 mg/kg day 2 to day 7) reduces inflammation (activated microglia, IL-1 β and TNF- α), white matter injury (oligodendrocyte progenitors and mature myelin) and neuronal injury (serotonergic neurons) in the neonatal brain (Buller et al., 2012; Wixey et al., 2012). In chronically hypoxic adult rats, ibuprofen (4 mg/kg daily for 7 days) was shown to block increases in the brainstem of the proinflammatory cytokines IL-1 β and IL-6. Exploring postnatal ibuprofen treatment in an IUGR animal model would be advantageous to assess its effects on reducing inflammation and preventing adverse neurological outcomes.

By reducing the numbers of activated microglia, using both minocycline and ibuprofen, both neuronal and white matter injury is decreased in the neonatal rat brain following HI (Wixey et al., 2011a, b, 2012; Buller et al., 2012). However, activated microglia can exist in two forms: M1 (toxic) and M2 (neuroprotective). Therefore, blocking all microglia may not be effective in preventing brain injury. Complete blockade of microglial activity has been shown to exacerbate brain damage in adult and neonatal HI injury models. Therapeutic interventions that specifically block M1 microglia have the greatest potential to protect the injured brain. Minocycline has been shown to selectively block M1 microglia. However minocycline has adverse effects when administered to neonates. Therefore, an alternative anti-inflammatory treatment is necessary for treating the neonate. Ibuprofen may be an ideal candidate. Determining whether ibuprofen can selectively inhibit the M1 activated microglial phenotype would also be beneficial for other neurological disorders where ibuprofen may be a useful treatment.

Conclusion: IUGR is the second leading cause of perinatal morbidity and mortality after prematurity, and occurs in approximately 5–12% of pregnancies. Few studies have focused on the mechanisms of brain injury in the IUGR neonate; information fundamental to the development of therapies to protect the IUGR brain. While treatment *in utero* is the preferred option, IUGR is often not evident until birth. The use of non-invasive *in vivo* techniques, such as magnetic resonance imaging (MRI) and electroencephalography (EEG) holds promise in characterising structural and functional aspects of myelination and connectivity in relation to injury progression, neuroplasticity and repair. Diffusion based MRI techniques such as diffusion kurtosis imaging (DKI) and neurite orientation dispersion and density imaging (NODDI) will enable more detailed and specific evaluation of white matter microstructure and integrity as well as cortical complexity.

Understanding the impact of inflammation in the IUGR brain is critical to improving long-term neurodevelopmental outcomes in these infants. Exploring interventions that specifically target inflammatory processes would not only directly reduce white matter and neuronal injury in the brain but may indirectly provide neuroprotection through effects on central and systemic inflammation. Are systemic inflammatory mediators infiltrating the brain or is inflammation originating in the brain and being released into the blood facilitated by BBB breakdown? Having the potential to detect early changes in blood biomarkers of brain injury is critical for early intervention and for determining the best possible treatments to prevent brain injury in IUGR neonates. These discoveries may also benefit other neurological disorders where BBB disruption occurs in concurrence with an inflammatory response such as neonatal HI, Alzheimer's disease and multiple sclerosis.

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Open peer review reports:

Reviewer 1: Han Zhang, University of North Carolina at Chapel Hill, USA.

Comments to authors: This is an excellent mini-review and prospective paper. It is well-written and easy to follow. The authors have great knowledge on possible neuromechanism of post-IUGR brain injury and neurological deficits. The possible treatments involving suppressing microglia and proinflammatory cytokines for anti-inflammation based mainly on animal studies are well summarized. Early detection based on blood biomarkers is also discussed. Possible early intervention options are discussed and compared, and the open question is raised to test the feasibility of Ibuprofen in future in the end.

Reviewer 2: Ahmed E Abdel Moneim, Helwan University, Egypt.

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