

Improving brain outcomes in the growth restricted newborn: treating after birth

Julie A. Wixey^{*}, Stella Tracey Bjorkman

Intrauterine growth restriction (IUGR) occurs when a baby is unable to grow normally due to receiving inadequate nutrients while developing in the womb. IUGR is a leading cause of perinatal death and long-term disability. The fetal brain is particularly vulnerable to IUGR conditions and adverse outcomes range from learning, attention and behavioral difficulties, to cerebral palsy. Due to medical advancements, more IUGR babies now survive, resulting in an even greater burden of disability. Many IUGR children require long-term medical care and support, and their families experience significant emotional stress and challenges. Currently there is no treatment available to protect the IUGR newborn brain and reduce these life-long burdens. While the development of strategies to reduce brain deficits in IUGR newborns have been proposed as in need of urgent research for several years, very few trials have been undertaken. Assessing long-term outcomes and thorough safety profiling of any treatment option is necessary prior to administering therapies to vulnerable newborns.

In IUGR newborns, changes in brain structure that occur during the prenatal period have been shown to persist into adolescence. Such changes alter brain connectivity and have been associated with neurodevelopmental disabilities. Our animal studies have demonstrated altered neuronal and white matter development both in fetal and postnatal brain (Kalanjati et al., 2017), with these alterations being associated with persistent inflammation even after birth (Wixey et al., 2019a, b).

Mechanisms of brain impairment in IUGR newborn: Multiple mechanisms have been shown to mediate cellular impairment in the IUGR brain. These include oxidative stress, excitotoxicity, necrotic and apoptotic degeneration, blood-brain barrier disruption and neuroinflammation. Recently, inflammation has been regarded a key mechanism associated with brain impairment in the IUGR newborn. Inflammation does not cease after the baby has been born and removed from the adverse intrauterine environment. In our large pre-clinical animal model of IUGR, we have shown sustained increases in activated microglia, astrogliosis, and increases in proinflammatory cytokines in the brain up to 4 days after birth (Wixey et al., 2019a). In humans, high concentrations of proinflammatory cytokines have been reported in the blood of preterm IUGR infants 2 weeks after birth (McElrath et al., 2013). Increases in proinflammatory cytokines in the blood have also been shown to correlate with adverse neurological outcomes at 2 years of age in preterm small for gestational age newborns (Leviton et al., 2013) suggesting that therapeutic targeting of inflammation following birth could reduce or even prevent significant ongoing neurological issues in these babies.

Treating the IUGR newborn to reduce neurological disorders: Treating the IUGR fetus *in utero* is likely to be beneficial, however 40% of IUGR babies are only diagnosed at or around the time of birth, with a much lower detection rate (average 18.7%) for low-risk populations (Gardosi et al., 2018). As our research has shown that inflammation-mediated brain injury in the IUGR newborn is sustained following birth, there is opportunity to treat the IUGR newborn to improve neurological outcomes. Treatments with anti-inflammatory properties such as melatonin, xenon, and erythropoietin have been investigated in clinical studies of hypoxic-ischemic encephalopathy however few clinical trials have explored such treatments in the IUGR newborn. Melatonin is being trialed antenatally in women with suspected IUGR fetuses, but there are no studies administering this treatment directly to the IUGR newborn. Currently, most research on potential postnatal treatments are in animal IUGR models, although studies are scarce. We have recently published some encouraging results from an anti-inflammatory postnatal treatment in the IUGR pre-clinical piglet model. Piglets are ideal animals to examine altered brain development arising from comprising perinatal events as the piglet brain growth spurt occurs during the perinatal period similar to the human. The piglet model of IUGR mimics many of the human pathophysiological outcomes associated with IUGR including asymmetrical growth restriction with brain sparing. Inadequate fetal growth in pigs is caused by alterations associated with placental insufficiency which is the most common cause of IUGR in the human population. Data obtained from the IUGR piglet model translates well to human IUGR pathology showing similarities in disturbances to both white and grey matter development (Kalanjati et al., 2017; Wixey et al., 2019a). Using the IUGR piglet as a model to trial neuroprotective treatments may indeed fast track translation to clinic.

Anti-inflammatory treatments for the IUGR newborn: Ibuprofen is a non-steroidal anti-inflammatory drug that works via inhibition of cyclooxygenase. It is widely used in the preterm newborn for the treatment of patent ductus arteriosus (PDA) and is safe and well tolerated with few reports of significant side effects. In the brain, ibuprofen crosses the blood-brain barrier enhancing cerebral blood flow autoregulation and oxygen delivery suggesting possible secondary neuroprotective benefits of ibuprofen use in the preterm. A recent study in extremely preterm infants treated with ibuprofen for PDA reported relatively preserved brain volumes, compared to untreated extremely preterm infants (Padilla et al., 2015). Ibuprofen treatment for PDA has also been shown to be associated with a reduction of intraventricular haemorrhage (Ohlsson and Shah, 2019).

We have recently shown a neuroprotective effect of ibuprofen treatment in the term IUGR newborn pig (Wixey et al., 2019b). Ibuprofen administered for three consecutive days from birth in the IUGR piglet, reduced neuronal and white matter disruption at postnatal day 4. We observed similar numbers of NeuN-positive neurons in the IUGR brain following ibuprofen treatment as in controls as well as recovery of neuronal morphology; in the untreated IUGR brain, neurons were smaller and displayed less defined neuronal labeling pattern. In the white matter, myelin tracts showed dense staining with luxol fast blue after ibuprofen treatment similar to the myelin tracts observed in the normally grown piglet brain whereas in the untreated IUGR brain, staining of myelin appeared patchy and less intense. Astrocyte density recovered and astrocyte and microglial morphology shifted to a less reactive inflammatory state suggesting a strong glial response to treatment (Wixey et al., 2019b). Ibuprofen had a strong anti-inflammatory effect in the IUGR brain as evidenced by a reduction in gene expression levels of key proinflammatory markers such as tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, IL-18, and C-X-C motif chemokine. The dosage and treatment used in this study is similar to that clinically indicated for the treatment of PDA in the preterm newborn; 20 mg/kg day 1 and 10 mg/kg day 2 and 3.

We also showed a significant reduction in apoptosis in the IUGR brain following ibuprofen treatment as determined by fewer activated caspase-3 labeled neurons (Wixey et al., 2019b). Recent studies have shown that ibuprofen extends its actions beyond that of an anti-inflammatory. In an *in vitro* and cell culture study using computational modelling and biophysical analysis, ibuprofen was found to directly inhibit caspase activation and prevent apoptosis (Smith et al., 2017). Caspases play a pivotal role in cell death and may represent a new target for ibuprofen, independent of its anti-inflammatory actions through cyclooxygenase inhibition. It is likely that inflammatory and caspase pathways are simultaneously modulated following ibuprofen treatment.

Stem cell therapy: Over the past decade, there has been increasing interest in the potential of stem cell therapy to improve brain outcomes for newborn brain injury. Studies have used several stem cell sources and types including neural stem cells, embryonic stem cells, bone marrow derived mesenchymal stem cells (MSCs), umbilical cord blood (UCB) stem cells, and human amnion epithelial cells.

MSCs have been the most extensively investigated due to their ability to modulate inflammation. MSC actions are mediated by a paracrine effect whereby they secrete protective factors in the body; however the cells are transient and thus effects may not last long-term. MSCs do not cross the blood-brain barrier and may be limited by lodgement/entrapment in the lungs when administered systemically.

In clinical trials of MSC therapy for stroke, limb ischemia, and multiple sclerosis, only mildly beneficial therapeutic effects have been shown. In newborn animal asphyxia models, several studies report significant neuroprotective effects of MSC use while other studies, report

no neuroprotective effects. Even though MSCs are one of the most promising stem cell types, different sources of MSCs (human amnion, UCB, placenta) as well as differences in cell isolation and preparation techniques (fetal versus maternal cells) could account for the inconsistency in pre-clinical and clinical studies.

Among the various sources of MSCs, UCB and placenta are the most promising because of their accessibility, ethical acceptability, and high proliferation capacity. Currently many studies are focusing on UCB to improve brain outcomes in newborn babies, however the effectiveness of UCB cells is limited by low available volume and cell numbers.

Only one study to date has examined UCB stem cells as a neuroprotectant after birth for treatment of the IUGR newborn. UCB stem cells were administered to fetal growth restricted newborn lambs 1 hour after life, followed by ventilation for 24 hours (Malhotra et al., 2020). Decreased neuroinflammation was reported as early as one day after administration of UCB stem cells (Malhotra et al., 2020). A reduction in the number of microglia in the brain was observed following treatment, however, UCB stem cells had no effect on the number of glial fibrillary acidic protein-positive astrocytes; altered glial cell morphology, which is indicative of an inflammatory response, was not reported. IUGR treated lambs also showed reduced levels of TNF- α in the blood in comparison to the non-treated IUGR lambs, however no significant differences were observed for any other cytokine measured. The minimal anti-inflammatory action of the stem cells may be due to the short time frame of brain examination following treatment, or stem cell efficacy as described above.

The placenta is an attractive alternative to UCB due to the greater availability and yield of stem cells. The placenta is rich in both MSCs and endothelial colony forming cells (ECFCs) and may provide significant and longer lasting neuroprotection. Unlike MSCs which are transient, ECFCs are long-lasting quiescent cells which engraft and only respond to injury when required. ECFCs circulate in peripheral blood and contribute to the repair and formation of new blood vessels. Up to 27 times more ECFCs can be obtained from the placenta than from UCB. In a hind limb ischemia model, combination therapy using placental ECFC/ MSC was superior to MSC therapy alone as a regenerative treatment to reduce limb loss or deformity (Sin et al., 2019). The authors also showed that these cells were not rejected in immunocompetent animal studies (Sin et al., 2019) demonstrating important evidence for use of these cells in allogeneic (off-the-shelf) situations. Whether this combination treatment could provide significant, long-lasting neuroprotection in the IUGR newborn remains to be determined. It is evident from preclinical and clinical trials that further research is required before we can find a definitive neuroprotective stem cell treatment for the newborn.

Curcumin: A naturally occurring molecule in the spice turmeric, has a wide range of pharmacological effects. Curcumin has significant neuroprotective properties that target inflammatory, oxidative, and apoptotic pathways; key components that are activated in the hypoxic cascade and can damage the

brain. Human and animal studies have proven curcumin extremely safe and well tolerated even at high doses. Recently, there has been an increase in the number of studies examining curcumin as a treatment for brain diseases, highlighting curcumin's potential neurotherapeutic actions.

A limiting factor for the successful application of curcumin as a neuroprotectant is its poor bioavailability and rapid metabolism by the liver. Delivering curcumin via nanoparticles may increase curcumin's solubility whilst improving curcumin's absorption, distribution, and metabolism.

A recent study using curcumin loaded nanoparticles in the postnatal day 7 neonatal hypoxic-ischemic rat showed it crossed the blood-brain barrier, diffused into the brain parenchyma and localised to regions of injury to provide a neuroprotective effect (Joseph et al., 2018). The authors reported a reduction in gross neuropathology and a change in microglial morphology suggestive of an anti-inflammatory effect (reduced activated state) at 3 days after curcumin treatment. To date there are no studies that have examined curcumin as a neuroprotectant in the IUGR newborn, however, a recent study assessed growth performance following curcumin supplementation for 24 days (26–50 days) in IUGR weaned piglets. Body weight significantly improved in IUGR piglets, with reduced levels of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) in the blood also reported. Research into curcumin treatment as a neuroprotectant in the newborn IUGR is warranted.

Summary: There is encouraging data from several studies of potential treatments for the IUGR newborn that particularly target inflammation which can be administered following birth. Further therapeutic developments could be guided by other neurological disorders in which inflammation is a key player. Availability of suitable treatments for the management of neonatal brain injuries will fundamentally impact the long-term neurodevelopmental outcomes for affected babies and result in enormous social, financial, and economic benefit for families and the greater community. Even though there is evidence therapies targeting inflammatory pathways protect the IUGR newborn brain, definitive long-term safety and efficacy data is required before embarking on clinical trials in this vulnerable newborn population.

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Julie A. Wixey*, Stella Tracey Bjorkman
UQ Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Herston, Queensland, Australia

*Correspondence to: Julie A. Wixey, PhD,
j.wixey@uq.edu.au.
<https://orcid.org/0000-0002-9716-8170>
(Julie A. Wixey)

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