Improving brain development in preterm infants with hypoxic-ischaemic encephalopathy

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The cause of preterm brain injury is undoubtedly multifactorial, however there is compelling evidence from large epidemiological studies that exposure to hypoxia ischemia during pregnancy or around the time of birth is a major risk factor.

Here we focus on our studies into the efficacy of two potential neuroprotective agents, magnesium sulphate and connexin 43 mimetic peptide, for hypoxic ischemic encephalopathy (HIE) in large animal translational models of preterm HIE.

Magnesium sulphate (MgSO4)

Background: MgSO4 has become widely recommended for perinatal neuroprotection in women at risk of preterm birth at <30 weeks of gestation. This recommendation was based on meta-analysis of randomized controlled trials that found a small but significant reduction in the risk of cerebral palsy and motor dysfunction in early childhood. However, subsequent follow up to school age suggests no significant improvement in neurodevelopmental outcome. These conflicting data indicate a need for rigorous testing of the efficacy of MgSO4 for HIE in the immature brain.

Aim: To test the neuroprotective efficacy of $MgSO_4$ for HIE in preterm fetal sheep, at a time when brain maturity is equivalent to that of a preterm infant at <30 weeks of gestation.

Method: At 104 ± 1 days (0.7) of gestation, fetal sheep were randomly assigned to receive a continuous infusion of i.v. $MgSO_4$ (n=7) or vehicle (control; n=10) starting 24 h before 25 min of asphyxia (*via* complete umbilical cord occlusion, to induce HIE), and continued for 24 h after occlusion. Fetal blood pressure, heart rate (FHR), carotid blood flow (CaBF), electroencephalography (EEG) and movement were measured continuously. 72 h after occlusion, fetal brains were processed for neuropathological assessment of the subcortical grey matter, including the mid-striatum, hippocampus, dentate gyrus and thalamus, and intragyral and periventricular white matter.

Results: $MgSO_4$ treatment increased fetal plasma magnesium levels from 0.78 to 1.89 mmol/L (P<0.05). The $MgSO_4$ infusion before asphyxia was associated with reduced FHR, EEG power and body movement (P<0.05 *vs* control). After asphyxia, MgSO4 reduced electrographic seizures but did not affect neuronal survival in subcortical grey matter, numbers of activated microglia and reactive astrocytes. $MgSO_4$ treated fetuses had reduced numbers of oligodendrocytes in the intragyral and periventricular white matter (P<0.05 *vs* control).

Conclusion: A clinically comparable dose of $MgSO_4$ was associated with reduced FHR, EEG power and body movement. $MgSO_4$ reduced electrographic seizures after asphyxia but did not reduce HIE and may increase oligodendrocyte loss.

Connexin 43 mimetic peptide

Background: In late preterm/term infants, basal ganglia injury after hypoxia-ischaemia remains a major contributor to poor neurodevelopmental outcomes such as cerebral palsy, learning disability and epilepsy. Opening of astrocytic connexin 43 hemichannels (Cx43), the constituents of gap junctions, during hypoxia-ischaemia is implicated in the pathogenesis of HIE.

Aim: To test the effect of Cx43 hemichannel blockade on the striatum, a major nucleus within the basal ganglia that is highly susceptible to HIE.

Method: A mimetic peptide that blocks connexin 43 hemichannels was infused into the lateral ventricle of chronically instrumented late preterm fetal sheep at 128 ± 1 days (0.87) of gestation. Short (1 h, n=6) or long (25 h, n=6) infusion of peptide or vehicle (n=6) was started 90 min after 30 min of cerebral hypoxia-ischaemia induced by reversible bilateral carotid artery occlusion. Fetal electroencephalography (EEG) was continuously monitored before, during and for 7 days after cerebral hypoxia-ischaemia. Fetal brains were collected for immunohistochemical assessment of striatal GABAergic neurons, including: calbindin-28k, calretinin, parvalbumin and glutamic acid decarboxylase (GAD) positive neurons.

Results: Hypoxia-ischaemia was associated with loss of calbindin 28k, calretinin, parvalbumin and GAD positive neurons (P<0.05 *vs* sham ischaemia). Short infusion of peptide did not improve survival of any striatal phenotype compared to ischaemia+vehicle. Long infusion of peptide was associated with increased survival of calbindin-28k, calretinin, parvalbumin and GAD positive neurons (P<0.05 *vs* ischaemia+vehicle). Improved survival of calbindin-28k, calretinin and parvalbumin positive neurons was associated with reduced seizure burden and improved recovery at 7 days after hypoxia ischaemia (P<0.05 *vs* ischaemia+vehicle).

Conclusion: Connexin hemichannel blockade after fetal cerebral hypoxia-ischaemia improved survival of striatal GABAergic neurons and was associated with reduced seizure burden and improved recovery. Collectively, these data suggest that blockade of connexin hemichannels has the potential to reduce basal ganglia injury after HIE.

All procedures were approved by the Animal Ethics Committee of The University of Auckland under the New Zealand Animal Welfare Act, and the Code of Ethical Conduct for animals in research established by the Ministry of Primary Industries, Government of New Zealand. Anaesthesia was induced by intravenous injection of propofol (5 mg/kg) and maintained using 2–3% isofurane in O2, by trained anaesthetic staff.