Experimental Models of Fetal Oxygen and Nutrient Deprivation: Implications for Brain Development

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Perinatal Hypoxia and Brain Injury: Overview

Clinical studies of perinatal hypoxic-brain injury
- intrapartum with acute hypoxic-acidemia
- antepartum with IUGR and/or nuchal cord

Animal studies of perinatal hypoxic-brain injury
- severe hypoxic-acidemia
- intermittent hypoxemia with cord occlusion
- chronic hypoxemia with IUGR

short and longer term brain development including neuroanatomic, neurometabolic and neurobehavioural outcomes
Perinatal Hypoxia and Brain Injury: Clinical Studies

Intrapartum hypoxic-acidemia

- Severe hypoxic-acidemia is associated with increased risk for HIE and later CP, although the majority of these infants will still be without noted complications.

- ACOG and AAP define severe hypoxic-acidemia sufficient to cause HIE +/- later CP as an umbilical art pH < 7.00 (incidence ~0.5%) or BE < -12 mmol/L (incidence ~2%) with ~20% of those infants showing neurologic sequellae.

suggesting a role for co-factors including GA at birth, duration of hypoxic-acidemia, fetal/newborn compensatory capacity, newborn resuscitation, infection
Perinatal Hypoxia and Brain Injury: Animal Study-Neurodevelopment

- Study in pre/near term primate and ovine fetus with sustained hypoxia, UCO insults, and cerebral ischemia leading to severe hypoxic-acidemia.
  - Brain injury characterized by necrotic and/or apoptotic cell death and variable with a predominance of injury in the subcortex-brainstem (anoxia studies, Windle 1950s), parasaggital cortex (prolonged hypoxia studies, Myers 1960s), or white matter-hippocampal/striatal (UCO studies, Mallard 1990s).
  - While relevant for modeling intrapartum hypoxia leading to brain injury, likely less relevant for modeling antepartum hypoxic insults.
Perinatal Hypoxia and Brain Injury: Animal Study-Neuromechanisms

Study in pre/near term ovine fetus with severe-hypoxic-acidemia reporting on mechanisms outlined for ischemic brain injury in adults with necrotic cell death.

- Necrotic cell death
  - membrane pump failure → cell lysis
    - primary with energy depletion
    - delayed with ↑ O₂ free radicals, ↑ excitatory AA, perfusion impairment, macrophage infiltration
Systemic and cerebral inflammatory response to
UCOs with worsening acidosis in the ovine fetus.
AP Prout 2010

– Repetitive UCOs over 2 to 4 hours, leading to
worsening acidemia with art pH to 6.90

- Fetal plasma IL-1β ↑ 2 fold at maximal fetal acidosis and
  early recovery indicating a systemic inflammatory response.
- Brain microglia and mast cell counts ↑ 2 fold at 24 hours
  recovery indicating a cerebral inflammatory response.
- Fetal systemic/cerebral inflammation triggered by placental
  hypoxia or hypoperfusion could be a contributing mechanistic
  pathway for brain injury with birth asphyxia.
The ovine fetal and placental inflammatory response to UCOs with worsening acidosis. A Xu 2015

- Repetitive UCOs over 2 to 4 hours, leading to worsening acidemia with art pH to ~ 7.00
  - Neutrophils were ↑ in the placenta at 48 h recovery.
  - Fetal plasma and AF cytokines were unchanged in normoxic-UCO animals, but ↑ in LPS-UCO animals.
  - Repetitive UCOs with severe acidemia can induce a placental inflammatory response and more so with simulated low grade infection and likely contributing to cytokine release in the umbilical circulation.
Brain injury and inflammatory response to UCOs is limited with worsening acidosis in the near-term ovine fetus. A Xu 2015

- Repetitive UCOs over 2-4 hours, leading to worsening acidemia with art pH to ~ 7.00

- Measures of brain inflammation (microglia/mast cell counts) and injury (necrosis/apoptosis) were unchanged in the normoxic-UCO animals, but TUNEL-positive cells were ↑ in the hippocampus of the LPS-UCO animals.

- Differs from UCO studies with more severe acidosis by Prout et al where brain inflammation ↑ and by de Haan et al 1997, where brain infarction and neuronal loss ↑, and animals became hypotensive.

- Likely a narrow threshold between hypoxic-acidemia with no sequelae and an insult causing cerebral impairment, with maintenance of blood pressure and thereby cerebral perfusion the critical issue here.
Perinatal Hypoxia and Brain Injury: Animal Study-CMR/State

Study in near term ovine fetus with varying hypoxic insult on CMR/behavioural state change

- **Moderate hypoxia 1 – 2 hrs, normal pH**
  - ↑ CBF, CMRO\textsubscript{2} maintained
  - ↓ FBM and EOG, ECoG unchanged

- **Severe hypoxia 6 – 8 hrs, severe acidemia**
  - ↓CMRO\textsubscript{2}
  - ↓↓ FBM and EOG, ↓ LV/REM and flattened electrocorticogram

- **Umbilical cord occlusion**
  - ↓↓ CMRO\textsubscript{2}, ↑↑ CMR glucose by 2 min
  - ECoG flattened by 90 sec
Near term ovine fetus

- Sustained hypoxia over 6 to 10 hours leading to worsening acidemia with art pH < 7.00

  CMR initially maintained by ↑ CBF and when this falls as MABP falls, by ↑ Fr O₂ extraction.

  Behavioural state activity shows a hierarchal response with FBM and EOG immediately ↓ (↓ fetal energy needs), whereas LV ECoG gradually ↓ (protecting the brain).

  With severe acidemia CMR falls and ECoG flattens likely reflecting synaptic transmission failure, but this is known to precede membrane failure with cell death, and is thereby likely protective by decreasing the brain`s energy needs.
Near term ovine fetus

- Severe intermittent 4 min UCO over 6 hours with profound hypoxemia and modest/severe acidemia

  Although CBF ↑↑, CMRO$_2$ unmeasurable by 2 min UCO while CMR glucose ↑ 3 fold and indicating a marked shift to anaerobic metabolism. These CMR alterations will result in an ~80% ↓ in energy production.

  ECoG flattened by 90 sec UCO likely indicating synaptic transmission failure, but with an ~50% ↓ in energy needs.

  Similar UCO study by C Mallard et al 1995, showed no neuronal cell loss in the cortex, with energy needs for membrane integrity likely maintained in the Kaneko study.

  The fetal brain demonstrates remarkable ability to protect cellular integrity by ↑ anaerobic metabolism and offloading non-essential energy needs. However, this disruption in energy-dependent processes may be detrimental to brain development with longer term hypoxic insult.
Perinatal Hypoxia and Brain Injury: Clinical Studies

Antepartum nuchal cord

- Variable FHR decels suggestive of cord compression are seen in ~5% of antepartum FHR recordings and have ↑ risk for nuchal cord.

- Infants with a nuchal cord at birth are smaller (increased IUGR) whereas their placentas are larger, which could involve chronic or intermittent cord compression.

- Infants with a symptomatic nuchal cord at birth have increased subclinical neurodevelopmental deficits, while the most common hypoxic condition associated with CP is the presence of a tight nuchal cord at delivery.

- Intermittent / chronic cord compression through the latter part of pregnancy may also impact the brain’s development.
Perinatal Hypoxia and Brain Injury: Clinical Studies

Antepartum IUGR

- IUGR is well associated with chronic hypoxemia (cord blood gases/cordocentesis), is a primary risk factor for HIE, and in turn for CP.
- IUGR +/- acidemia at birth, may also lead to other neurologic sequelae including cognitive impairment, attention deficit disorder and possibly schizophrenia and autism.
- Chronic fetal hypoxia with IUGR through the latter part of the pregnancy may also impact the brain’s development.
Perinatal Hypoxia and Brain Injury: Animal Study-Neurodevelopment

Study in pre/near term ovine and guinea pig fetus with less severe, but chronic hypoxia.

- Intermittent umbilical cord occlusion
  - Necrotic cell death – minimal
  - Apoptotic cell death – minimal
  - Intermediate filament proteins ↓
  - synapse proteins altered

- Intrauterine growth restriction/chronic hypoxia
  - Apoptotic cell death ↑
  - Myelination ↓
  - Dendritic morphology altered
Perinatal Hypoxia and Brain Injury: Animal Study-Neuromechanisms

Study in pre/near term ovine and guinea pig fetus with less severe, but chronic hypoxia.

- Intermittent UCO/IUGR/chronic hypoxia
  - altered behavioural state activity
  - altered growth factors
  - ↑ pro-apoptotic factors
  - ↑ oxidative stress
  - inflammation
  - ↓ protein synthesis
Pre/near term ovine fetus

- Intermittent 90 sec UCO every 30 min for 3-5 hours daily over 4 days with acute, but limited hypoxemia and no cumulative acidemia

- PT/NT animals, very low levels of necrotic-appearing cells across all brain regions. E Rocha 2004

- PT/NT animals, very low levels of apoptotic-appearing cells across all brain regions. A Falkowski 2002

- PT but not NT animals, ↓ intermediate filament proteins in gray matter and white matter. E Rocha 2004

- PT animals, ↓ SYN in most brain regions; NT animals, ↑ MAP-2 in CA1 and thalamus. M Czikk 2014

- PT animals, ↓ BDNF and TrkB in all brain regions; NT animals, ↑ BDNF in CA1 and 3. H Nishigori 2008

- NT animals, flattened ECoG by 90 sec of UCO and ↓ LV/REM ECoG state activity. Y Kawagoe 1999
Figure 6. High-power sections (×63) from the hippocampus CA1 of preterm (A) control and (B) UCO group animals showing BDNF immunoreactivity and of preterm (C) control and (D) UCO group animals showing TrkB immunoreactivity in the pyramidal cells and surrounding neuropil and the decrease with UCO insult. BDNF = brain-derived neurotrophic factor; TrkB = tyrosine kinase receptor; UCO = umbilical cord occlusion.
Near term guinea pig fetus

- Uterine artery ligation/ablation at mid-gestation leading to fetal growth restriction

- FGR fetuses exhibited reduced synaptogenesis, synaptic maturation, and myelination primarily in the hippocampus and associated efferent tracts.

- Neurodevelopmental changes were more pronounced in sFGR compared to aFGR animals.

- Altered hippocampal development involving synaptogenesis and myelination may be a mechanism by which cognitive deficits manifest in growth restricted offspring.

- Substantial fetal demise rate leading to variable FGR and likely due to the abrupt nature of uterine artery ligation/ablation.
SYN = pre-synaptic vesicle protein marker
SYNAP = post-synaptic dendritic protein marker

K Piorkowska 2014
Near term guinea pig fetus and offspring

- Moderate maternal nutrient restriction (MNR) at 70% of the control diet pre-pregnancy, switching to 90% at mid-pregnancy

Placental structural abnormalities and aFGR with increased brain/fetal wt which was greater in males than females as also reported with FGR in humans (Kramer MS 1990), and suggesting less restriction in brain growth.

FGR-MNR fetuses had higher levels of HP-1 in most brain regions, as a widely used marker for tissue hypoxia and indicating lower levels of oxygenation which was again greater in males than females.

The lower level of brain oxygenation in FGR-MNR males may relate to the O$_2$ consumptive needs of continuing brain growth in males vs adaptive growth restriction in females and contribute to sex-specific expression of later adverse neurodevelopment.
Growth measures from select AGA-Control and FGR-MNR fetuses undergoing full necropsy (> and < 80g, respectively), presented as mean % change from control values.

AA Elias 2016
Photomicrographs (40X) illustrating cell staining for HP-1 (brown) in figures A and B, and with the binary colour threshold setting used for cell counting overlaid in figures C and D, with cells above threshold (green) counted as positive.
HP-1 positive cells/mm² for the brain regions of the AGA-Control and FGR-MNR fetuses.

* p < .05
** p < .01
*** p < .001

Y Maki SRI 2016
HP-1 positive cells/mm² for the brain regions of the male and female AGA-Control and FGR-MNR fetuses.

Y Maki SRI 2016
Perinatal Hypoxia and Brain Injury: Clinical & Animal Study

Questions Raised/Future Study

- Study of severe birth asphyxia indicates the brain`s protection against cell death by ↑ CBF, Fr O₂ extraction, and anaerobic metabolism, and ↓ non-essential energy needs, but likely with a narrow threshold between no injury, impairment, and death.

- Study of less severe hypoxia over longer antenatal periods indicates that growth processes in the brain may be altered including synaptogenesis/myelination and ↑ apoptosis, but limited correlation to neurobehaviour outcomes.

- Limited study of confounders in cerebral responses to hypoxia, e.g. oxidative stress/inflammation.
Maternal Nutrient Restriction - IUGR

A. Ghaly et al, NDN Meeting 2014