Novel therapeutic strategies in maternal inflammation induced brain injury and cerebral palsy

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Introduction

- Injury to the developing brain is unique: different responses based on the timing of injury

- Maternal intrauterine infection implicated in brain injury resulting in cerebral palsy and neurodevelopmental disorders such as autism

- Significant correlation: chorioamnionitis and cerebral palsy; maternal infection and autism.

- Injury involves both grey matter and white matter.

- Motor, somatosensory and cognitive deficits noted in CP
Maternal Infection and FIRS
Mechanism of brain injury

IL-1β; TNF-α; IL-6; IL-8

Periventricular leukomalacia

Activated Microglia

Increased PBR on mitochondria of microglia detected using of [11C](R)PK11195

Animal Model

Pregnant New Zealand White rabbits (28 days)
Laparotomy and intrauterine injection

(Maternal serum for cytokines
0,2,6,24,48 hrs)

Saline

LPS (20µg/Kg) from E. Coli
(Born spontaneously at term-31 days)

Control kits

Endotoxin kits

Neurobehavioral scoring, PET scan, MRI, and/or immunohistochemistry
Inability to maintain prone posture, hypertonia, and impaired locomotion noted in endotoxin kits; p<0.01 (Scoring for newborn rabbits based on Derrick et al. 2004)

S. Kannan group et al. AJOG; 2008
Is this phenotype associated with presence of activated microglia in the neonatal brain?
PET scan

$[^{11}\text{C}]\text{PK11195= PET tracer (300-500 } \mu\text{ci)}$

- IV
- Control and Endotoxin kits
- Customized head holders with markers for MRI and PET
- Scan for 60 min
- MRI for co-registration
Co-registration of PET and MRI images done with markers seen on both.

3D Region of interest drawn for the cerebrum and midbrain.

SUV plotted over time and slopes and intercepts compared between groups.

SUV = Activity in ROI (μCi/g) x Wt (g)

Injected dose (μCi)
Detection of activated microglia by PET scan
$^{11}$C PK11195 uptake in the neonatal rabbit brain

Increase in tracer retention seen over time in the endotoxin group indicating specific binding of the tracer to activated microglia
Activated Microglial Cells

Change in microglial morphology from ramified to more amoeboid and rounded form with endotoxin exposure.

Increased activated microglia in white matter tracts in endotoxin kits.

(Kannan S group et al JNM, 2007; Journal of Child Neurology, 2009)
Activated microglia and oligodendrocytes

A decrease in the number of mature oligodendrocytes (MBP staining) is noted with an increase in the presence of activated microglia in endotoxin kits; IC=Internal Capsule
Decrease in Myelin basic protein staining noticed on postnatal day 5 in the corpus callosum, corona radiata and internal capsule
Involvement of somatosensory cortex and neuronal injury
Tryptophan metabolism in the brain

L-Tryptophan → Serotonin (TH)

L-Kynurenine → Kynurenic acid

3 hydroxy-kynurenine

Quinolinic acid

Excitotoxic Injury/ Seizures

Tryptophan metabolism can be evaluated by PET using 11C AMT as a tracer
Serotonin in the newborn rabbit brain

**11C AMT metabolism**

- Decreased serotonin concentration in the cortex and hippocampus
- Tryptophan metabolism along non-serotonin pathway in PVR (Kynurenine pathway)

**Serotonin concentration**

*Kannan S et al., In press, JCBFM 2010.*
Serotonin fibers in the somatosensory cortex

**5-HT staining**

- A decrease in 5HT staining fibers noted in layer IV of the somatosensory cortex.
- 5HT fibers project from serotonergic neurons in raphe nucleus and from thalamus.
- Thalamocortical fibers transiently express 5HT transporter (5HTT) during development.
- Decrease in 5HTT expression indicates loss of thalamocortical fibers.
Increased apoptotic cells seen in VP thalamus of endotoxin kits when compared to controls on day 1 of life.

This may result in the loss of thalamocortical afferent fibers that transiently express 5HTT.

Decrease in fibers may be a result of direct injury to the fibers and/or loss of thalamic neurons that project to the sensory cortex.
Injury to dendrites

- Impairment in dendritic branching, organization and decreased spines seen in endotoxin kits upon Golgi staining.
- Associated with learning deficits and memory impairment
- Seen in brains of patients with mental retardation

Determine if there is impairment in learning associated with this injury

Molecular markers responsible for synaptogenesis, dendrite formation and axon guidance
Longitudinal PET Measure of Microglial Activation

The slope decreases over time with the greatest drop (decrease in slope) after day 4 indicating a decrease in the number of activated microglial cells.
Conclusions

- Maternal endotoxin exposure induced microglial activation is associated with a phenotype of cerebral palsy in the rabbit model.

- PET imaging of microglial cells may be used as a diagnostic tool to determine the presence of neuroinflammation in the neonate.

- Maternal inflammation is associated with decreased serotonin in the cortex with injury to thalamocortical afferents. May explain somatosensory impairment in CP.

- Targeting activated microglia with anti-inflammatory agents may help in attenuating injury and improving deficits.
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