Neuroinflammation: Imaging and targeted therapy

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Magnitude of the problem

- Perinatal brain injury—a major cause of morbidity and mortality

- 1 in 303 children have cerebral palsy or 3.3 per 1,000 8-yr old children have cerebral palsy (CDC)

- 1 in 110 children have autism spectrum disorders (CDC, 2009)

- Lifetime costs for an individual with cerebral palsy is about $921,000.

- Unmeasurable social and emotional costs.
Magnitude of the problem

- Wide spectrum of clinical presentations with perinatal brain injury
- Injury to the developing brain is unique: different responses based on the timing of injury
- Injury involves both grey matter and white matter.
- Motor, somatosensory and cognitive deficits noted in CP
- Difficult to study, animal models not representative
- Diagnoses is clinical and often late
Inflammation and brain injury

- Immune dysregulation of the brain implicated in autism and cerebral palsy

- Significant correlation between chorioamnionitis and PVL/cerebral palsy (Dammann, Wu, Yoon)

- Increased incidence of autism in patients with cerebral palsy (Kirby, 2011)

- Autism may be related to maternal immune activation, infection/inflammation (Patterson, Fatemi, Meyer, Shi)

- Immune activation may be mediated by microglia in the fetal/newborn brain
Glia: The neglected brain cells?

Glial cells are more than “nerve cement”

Glial cells make up ~90% of the brain cells and more than half the volume!

As we go up in the evolutionary cycle, more of the brain is made of glia

Fruit Fly: 25%
Mouse : 65%
Human: 90%

For every neuron there are 9 glia!

http://stanmed.stanford.edu/2009fall/article6.html [article by Bruce Goldman (Stanford)]
“Neuro” Science versus “Glia” Science

Santiago Ramon y Cajal

Shared Nobel Prize in Physiology in 1906; ‘Fathers’ of the field of neuroscience

Camillo Golgi

Over the last several years the focus has shifted to microglia and astrocytes for preservation of neurons; Ben Barres, JD Rothstein etc.
Microglial Cells: Unique Role in the developing brain

- Resident macrophages in the brain; “Surveillance Cells”
- Undergo dynamic changes in the developing brain
- Present in the white matter tracts in the developing brain in high density (Monier, 2007)
- Decrease in numbers and move to the cortex from the white matter tracts by 1-2 years of age (Billiards, 2006)
- Increased presence of microglia noted in the brain of patients with PVL and autism (Haynes, 2003; Vargas)
- Play a role in remodeling
- Activated in the presence of inflammation
Fetal Inflammatory Response Syndrome

IL6

Courtesy Dr. Roberto Romero, Perinatology Research Branch, NICHD
Maternal Inflammation and CP
Mechanism of brain injury?

Animal Model

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

- 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
Rabbit model of cerebral palsy

Control Day 1
Jumpy jerky movements

Endotoxin pup 1
Righting reflex

S. Kannan group et.al. AJOG; 2008
Detection of activated microglia by PET scan
An imaging biomarker for neuroinflammation

(Kannan S et al JNM, 2007; Journal of Child Neurology, 2009; Devl Neuoscience, 2011)
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)
White matter injury
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
Injury to neurons

- 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
Corresponds with epidemiological studies where inflammatory cytokines are seen in the postnatal serum up to at least 2 weeks of age in neonates who later develop CP.

ELGAN Studies: 2011; 2012

There may be window of opportunity to treat even after birth
BBB is a major challenge for drugs and delivery vehicles

Targeting ‘diffuse’ neuroinflammation/microglia

Even if the vehicle is transported, can it accumulate in enough amounts to create a therapeutic effect?

The brain injury has already occurred in utero. Can the motor deficit improve?
Dendrimers: ‘Tree-like polymers’
In collaboration with Kannan Rangaramanujam
Co-Director, Center for Nanomedicine, Wilmer Eye Institute

Dendrimers are well-defined, tree-like polymers made synthetically, with a size of ~ 4 – 20 nm.
Flexible, open structure, where each component of the tree can be manipulated
Biocompatible, can be made biodegradable
Multifunctionality (therapy, imaging, targeting)

Strategy: use the intrinsic targeting and release properties of dendrimers as building blocks and tailor the nanodevice to the specific clinical application
Biodistribution of dendrimer in newborns with CP (Subarachnoid)

Dendrimer localizes to activated microglial cells in the brain of kits with neuroinflammation, far removed from the site of injection.
Dendrimer localizes in activated microglia and astrocytes even upon IV administration.

Kannan S et al, Science Translational Medicine, 2012
But can it release the drug specifically where we want it to?

- N-acetyl cysteine has anti-inflammatory and anti-oxidant effects; GSH precursor
- Has been shown to reduce infarct volume and inflammation in animal models of stroke and cerebral ischemia
- NAC conjugated to dendrimer such that it will not release in plasma but will release intracellularly in a sustained manner
- Validated in vitro

Internalization
Delivery of the Drug

Expulsion

PAMAM Dendrimer

S-S-NAC

Cleavable disulfide bond

GSH

NAC released

Kannan et al US patents filed

**NAC linked by disulfide bond**

**Internalization**

1. **Cleavable disulfide bond**
2. **GSH released**
3. **NAC released**

**Expulsion**
Neurobehavioral Assessment
CP Kit-PBS treatment on day 1

Kannan S et al, Science Translational Medicine, 2012
Neurobehavioral Assessment
CP Kit- D-NAC 10mg/kg Day 1

Kannan S et al, Science Translational Medicine, 2012
Dramatic Improvement in motor function seen by Day 5, upon dendrimer-NAC treatment

Kannan S et al, Science Translational Medicine, 2012
Decrease in activated microglia
Myelination and neuronal injury

- Associated with decrease in markers of oxidative injury
- Increase in glutathione levels
- Decrease in inflammation at day 5 of age
- Decrease in neuronal injury

Kannan S et al, SciTM 2012
Summary

• **Postnatal therapy for prenatal injury**

• **Targeted therapy can prevent or arrest fetal neuro-inflammation**

• **Platform for delivering drugs in a targeted, sustained manner for brain injury: implications in other neurodegenerative diseases**

Ongoing Studies

• Can we switch the microglial phenotype to prevent ongoing injury and promote resolution?
• Effect of neuroinflammation in altering serotonin and kynurenine metabolism in the developing brain
• Targeting the kynurenine pathway in microglia to decrease injury and facilitate cortical development in the perinatal age
• Targeting specific enzyme pathways that are seen primarily in activated microglia
• In vitro slice studies to evaluate microglial function and action and uptake of nanoparticles/dendrimers with change in phenotype
• Evaluation of neuroinflammation and dendrimer therapies in models of TBI, glioblastoma, EAE etc.
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- Hypothermic Cardiac Arrest (Baumgartner group)

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