Targeted, systemic nanotherapies for neuroinflammation- approaches for CNS disorders

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Central Nervous System (CNS) diseases

CNS diseases account for 11% of the global burden of disease → $400 billion annually

Diffuse nature of CNS diseases makes treatment challenging
- Widespread impact on neuronal and glial cells

Therapeutics are largely excluded from the CNS due to:
- Drug delivery/permeability across the blood-brain barrier
- Penetration of therapeutic agent within brain parenchyma

Neuroinflammation plays key role in pathology of many CNS diseases: Alzheimer’s, Stroke, Multiple Sclerosis, Autism, Traumatic Brain Injury, Gliomas, Retinal degeneration (AMD)

Omidi and Bara (2012) BioImpacts
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%

Behind every neuron there are 9 glia!

http://stanmed.stanford.edu/2009fall/article6.html [article by Bruce Goldman (Stanford)]
Microglia and Astrocytes

- **Microglia**: Microglia are *immune system cells* that act as the first and main form of active *immune defense* in the central nervous system (CNS). They defend the brain and spinal cord, constantly excavating the CNS and attacking and engulfing infectious agents.

- **Astrocytes**: (a) *regulation of cerebral blood flow* – their activation dilates blood vessels and the endfeet have been observed to be intimately associated with blood vessels.  
  (b) *Signal transduction between synapses*  
  (c) *Neuro-glial transport*
Emerging literature suggests that neuroinflammation and activated microglia are central players in many neurodegenerative diseases.
Our Approach

Controlling/engineering the behavior of activated microglia and astrocytes

Can we

(1) Access them?

(2) Calm them down and prevent ‘destruction’?

(3) Switch their phenotype and ‘resolve’ inflammation?
Overcoming barriers for delivery to the brain

1. Overcome the blood-brain barrier
2. Move within diseased brain tissue parenchyma
3. Uptake into specific disease associated cells

Neuroinflammation, mediated by microglia and astrocytes, has recently been elucidated as a major player in many brain diseases and impacts all barriers listed above.

Nanodevices can overcome these barriers, if appropriately engineered based on disease.

Challenges

- 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?
- In many neurodegenerative diseases, the brain injury occurs well before diagnosis/detection. Can the damage still be reserved?
What dictates *in vivo* brain distribution and cell-specific uptake of nanoparticles in CNS disorders?

- Nanoparticle properties
- Disease etiology
- Developmental age
- Animal model
Dendrimers

Hydroxy-terminated generation-4 PAMAM (‘neutral’) dendrimers

- Non-cytotoxic, cleared in tact
- Beta-alanine repeat units (peptide-like)
- Amide-amine-OH desirable for intracellular pharmaceutics

Strategy: Use the unique interactions between PAMAM dendrimers and disease pathology: no ligands
Targeting Neuroinflammation In Cerebral Palsy

(with Sujatha Kannan, Associate Professor Anesthesiology and Critical Care, Johns Hopkins School of Medicine)

Collaborators

- Michael Johnston
- Ali Fatemi
- Barbara Slusher
- Mary Ann Wilson
- Mary Blue
Neutral PAMAM dendrimer rapidly co-localizes in activated microglia in regions affected by cerebral palsy.

No uptake in the subventricular zone (SVZ) that is predominantly comprised of neuronal progenitors.

D-OH is found in regions with BBB impairment and significant microglia activation.

Nance et al., In preparation
The Approach: Overcoming the totality of the problem

1. Overcome blood-brain barrier in the injured brain

Dextran FITC (70kDa)

Blood vessel

20nm Nanoparticle

Blood vessel

4nm Dendrimer (14kDa)

Cellular

2. Move within diseased brain tissue parenchyma

3. Uptake into specific disease associated cells

Nanodevices can overcome barriers within the brain, if appropriately engineered based on disease

Nance et al In preparation
Dendrimer brain uptake/efficacy in a canine brain injury following hypothermic cardiac arrest model

Dendrimer localizes in injured neurons and activated microglia. Combination therapy with dendrimer is effective with 10-30-fold less drug, and has significantly less side effects, compared to free drugs.

Collaborators:
- William Baumgartner
- Michael Johnston
- Mary Ann Wilson
- Sujatha Kannan
- Mary Blue

Large Animal model – 30 kg
Dendrimer-FITC

Mishra et al. (JHU Team) ACS Nano (2014)
Can this be used to deliver therapeutics to target cells?
Synthesis of Dendrimer-N-acetyl Cysteine Conjugate

- Payload: 20 NAC molecules per dendrimer
- size = 20,085 Da, 5 nm
- Highly soluble in water

References:
- J. Controlled Release, 2010, 142, 447
- Y.E. Kurtoglu et al, Biomaterials, 2009, 30, 2112
- B. Wang et al, Int. J. Pharm 2009, 377, 159
Neurobehavioral Assessment
Post-Natal Evaluation – **Day 1**

CP kits:
- PBS

CP kits:
- Dendrimer-NAC

Littermates
Significant motor function improvement: Neurobehavioral Evaluation – **Day 5**

- **CP kits:**
  - PBS
  - Dendrimer-NAC

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**Littermates**

**Single dose on Day 1**

Significant improvement in motor function with Dendrimer-NAC therapy

Dramatic Improvement in motor function seen by Day 5, upon Dendrimer-NAC treatment

- Patents Pending/awarded (2009/2010);
- Science Trans. Med (April, 2012);
Large dose of free NAC appears to attenuate inflammation, but does not produce motor function improvement. Targeted delivery to activated microglia and astrocytes appears to be key to motor function improvement in CP.

Kannan et al., Science Trans. Med (April, 2012);
Intravenous dendrimer therapies for AMD, retinal disorders

-In collaboration with Gerard Lutty Group

Wilmer Eye Institute, Johns Hopkins
Systemic Dendrimer-NAC therapy: Early AMD

~80% suppression of neovascularization and inflammation

Manuscript in preparation (2014)
Patent filed (2014)
Systemic dendrimer-NAC therapy: Late AMD

Systemic dendrimer therapy can cause CNV regression, and attenuate pro-inflammatory response in retina and choroid.

Attenuation of pro-inflammation in the retina

Manuscript submitted (2014)
Patent filed (2014)
Conclusions

1. Neuroinflammation, associated with activated microglia and astrocytes can be a therapeutic opportunity -

2. Dendrimers, the 5nm squishy nanostructures, have unique \textit{in vivo} cellular biodistribution in CNS as a function of pathology (targeting neuroinflammation) in multiple animal models of CNS disorders

2. Taking advantage of the structural and functional aspects of dendrimers can lead to improved targeted therapeutic applications in challenging CNS disorders
   -(Cerebral Palsy, AMD, Stroke, Brain injury following HCA).

3. Multifunctional CNS therapeutic and imaging platform
Development and validation of tailored delivery systems in appropriate animal models, with a goal of translation.
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