Effect of rolipram-loaded polymeric micelle nanoparticle on cAMP level in hypoxia condition and in rat SCI model

1Christian Macks, 1So Jung Gwak, PhD, 2Michael Lynn, MD,
1JeoungSoo Lee, PhD.
1Bioengineering, Clemson University, Clemson, SC,
2Department of Neurosurgery, GHS, Greenville, SC
Spinal Cord Injury
*200,000 people in the US are currently living with spinal cord injury
*12,000-20,000 new cases each year in US
* chronic pain, spasticity, respiratory impairment, loss of bowel or bladder control, and sexual dysfunction

Traumatic brain injury
* 2.5-6.5 million people in the US are currently living with traumatic brain injury
* **Physical symptoms**:
  - Loss of consciousness, Persistent headache, Repeated vomiting or nausea, convulsions or seizures, weakness or numbness in fingers and toes, loss of coordination
* **Cognitive or mental symptoms**:
  - Profound confusion, agitation, combativeness or Coma
* 1.5-2 million new cases in the US each year
* physical, psychological and economical consequences

No effective pharmacological therapy
Obstacles to CNS Nerve Regeneration

• **Extrinsic Factors**
  - Primary injury
  - Secondary neuronal cell death
  - Destruction of physical structure of axons and supporting cells
  - Formation of cystic cavities at lesion site
  - Absence of neurotrophic / survival factors
  - Absence of growth-promoting adhesion ligands
  - **Exposure to myelin-associated inhibitory glycoproteins**
  - **Glial scarring - increased expression of inhibitory proteoglycans**

• **Intrinsic Factors**
  - Inability to sustain expression of regeneration-associated genes
  - **Down-regulation of cAMP/PKA signaling pathway**

*Geller and Fawcett, 2002; Scwab 1990; McKeon at al. 1991; Fitch et al. 1999.*
Three myelin associated molecules termed Nogo A, MAG (myelin associated glycoprotein), and OMgp (oligodendrocyte myelin glycoprotein) have been identified as inhibitory substrates for axon growth and function.

MAG, Nogo A, and OMgp bind to the Nogo-66 receptor (NgR) and inhibit axon growth and function.

CSPGs have also been shown to activate the RhoA/ROCK signaling pathway.

cAMP
- activate PKA and stimulate cell survival genes through CREB
- Downregulates pro-inflammatory cytokines

Scheme 1. Myelin-associated and glial inhibitors and intracellular signaling pathway
Neuron-specific nanotherapeutics

Three functional components:

1. **anti-NgR1 antibody**: to deliver the nano-therapeutics to neurons and interfere with the function of existing NgR1 receptors by antagonizing the binding of myelin associated inhibitors.

2. **RhoA siRNA**: to target common intracellular signal transduction pathways for both myelin and CSPGs.

3. **Rolipram, a phosphodiesterase inhibitor**: to increase intrinsic neuronal growth capacity by preventing injury-induced reductions in cAMP levels and suppress expression of pro-inflammatory cytokines and other mediators of inflammation.
Long-term goal: Neuron-specific multifunctional nanotherapeutics

Scheme 2. Antibody functionalized polymeric micelle nanotherapeutics
Synthesis of PLGA-g-PEI (PgP)

Synthetic route of PLGA-g-PEI (PgP)

$^1$H-NMR spectrum of PLGA-g-PEI (PgP)
Cationic Polymeric Micelle as a RhoA siRNA Carrier for Axonal Regeneration in Rat SCI model

INTRODUCTION

The regenerative capacity of the injured spinal cord is extraordinarily limited due to both intrinsic neurologic and extrinsic injury-related factors that probably act synergistically in preventing effective neural repair. A number of strategies have been developed that involve pharmacological, mechanical, or growth factor-based approaches to promote axonal regeneration, and all these therapies are relatively non-specific and cause significant side effects. We have previously reported that systemic enhancement of spinal cord regeneration by intrathecally delivered Cationic Polymeric Micelle (CPM) was due to the delivery of a high dose of Brattleboro spinal cord regeneration-promoting factors. In this study, we investigated the ability of CPM to enhance the delivery of siRNA to the rat spinal cord and promote axonal regeneration in a rat spinal cord injury model.

METHODS

Synthesis and characterization of CPM: Poly(ethylenimine-g-poly(2-aminoethylglycine) 8000)

1. By synthesis, Cationic Polymeric Micelle (CPM) was preformed in vitro with high siRNA-loading efficiency and enhanced cellular uptake of siRNA.

2. CPM-siRNA complexes were evaluated in vitro and in vivo.

Characterization of CPM-siRNA complexes

1. CPM-siRNA complexes were evaluated in vitro and in vivo.

2. CPM-siRNA complexes were evaluated in vitro and in vivo.

3. CPM-siRNA complexes were evaluated in vitro and in vivo.

4. CPM-siRNA complexes were evaluated in vitro and in vivo.

RESULTS

1. CPM-siRNA complexes were evaluated in vitro and in vivo.

2. CPM-siRNA complexes were evaluated in vitro and in vivo.

3. CPM-siRNA complexes were evaluated in vitro and in vivo.

4. CPM-siRNA complexes were evaluated in vitro and in vivo.

5. CPM-siRNA complexes were evaluated in vitro and in vivo.

6. CPM-siRNA complexes were evaluated in vitro and in vivo.

CONCLUSIONS

1. CPM-siRNA complexes were evaluated in vitro and in vivo.

2. CPM-siRNA complexes were evaluated in vitro and in vivo.

3. CPM-siRNA complexes were evaluated in vitro and in vivo.

4. CPM-siRNA complexes were evaluated in vitro and in vivo.

ACKNOWLEDGMENTS

1. CPM-siRNA complexes were evaluated in vitro and in vivo.

2. CPM-siRNA complexes were evaluated in vitro and in vivo.

3. CPM-siRNA complexes were evaluated in vitro and in vivo.

4. CPM-siRNA complexes were evaluated in vitro and in vivo.

5. CPM-siRNA complexes were evaluated in vitro and in vivo.

6. CPM-siRNA complexes were evaluated in vitro and in vivo.
Rolipram loading in PgP (Rm-PgP) by solvent evaporation method

- % Rolipram loading efficiency: (Rm loaded in PgP / Rm added in PgP) × 100
- ~86% in PgP (1 mg/ml)
- *P<0.05 compared to water
Effect of Rm-PgL on cAMP level in rat Cerebellar Granular Neuron (CGN) cells cultured in hypoxia condition

- Dose of Rm: 10 µg /well
- *P<0.05 compared to normoxia
Effect of Rm-PgP on CGN cell survival under hypoxia

Immunofluorescent staining for beta III tubulin in CGN cells cultured in (A) normal oxygen conditions, (B) hypoxia conditions with untreated, (C) PgP only treated, (D) Rm-PgP treated, and (E) Rm-DMSO treated. 100x magnification
Effect of Rm-PgP on neurite length in rat Cerebellar Granular Neuron (CGN) cells in hypoxia condition

- Dose of Rm: 10 µg/well
- *P<0.05 compared to normoxia
Rm-PgP effect in rat SCI model

- Generation of compression spinal cord injury model

T9 spinal cord
Localization of DIR- PgP/pDNA polyplex after local injection in rat SCI model

Rat: Sprague Dawley (70-100 gm), 10 µg pDNA/rat
Effect of Rm-PgP on cAMP level in rat SCI model

- Rm-PgP: 1µg Rolipram was loaded in PgP (1 mg/ml) solution
- Dose: 10 µg, 10 µl (1 mg/ml) Inj. by Hamilton Syringe (26 G)

*P<0.05 compared to sham

Groups (n=6)
1. Sham control
2. SCI-Untreated
3. SCI- PgP control
4. SCI-Rm-loaded PgP
Effect of Rm-loaded PgP on apoptosis at 3 days post-injection in rat SCI model

<table>
<thead>
<tr>
<th>TUNEL</th>
<th>DAPI</th>
<th>Merged</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rm-PgP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Original magnification: 50X
Anti-inflammatory activity of Rm-loaded PgP in rat SCI model

Original magnification: 50X
Summary & Ongoing Study

- Rm-PgP restores cAMP levels and increases neurite outgrowth in *in vitro* hypoxia injury model.
- Rm-PgP exhibits prolonged residence time after injection and reduces apoptosis and inflammation in rat compression SCI model.
- We are studying Rm-PgP effect on pro-inflammatory and anti-inflammatory cytokine expression *in vitro* and *in vivo*.
- We are studying the synergistic effect of Rm-PgP/RhoA siRNA nanotherapeutics on functional recovery in rat compression SCI model.
Thank You!!!

4D lab Members:
So-Jung Gwak, PhD
Da Un Jeong, PhD
Angela A. Bryant, PhD
Graham Temples
Christian Macks
Michael DiBalsi
Ben Green
Breanne Hourigan
Erica Beal
Noah Cecil
Joseph Whitaker
Justin Nice, MS
Jeremy Zhang, PhD

Collaborators:
Dr. Ken Webb in Bioengineering
Dr. Mark Kindy in Neuroscience, CSFU
Dr. Naren Banik in Neuroscience, MUSC
Dr. Michael Lynn, Neurosurgeon in GHS

Funding:
1. SC COBRE NIH grant # P20GM103444 from NIGMS/NIH
2. SC-Spinal Cord Injury Research Fund Grant #2014 I-02

COBRE Bioengineering and Bioimaging Core
Dr. Guzelia Korneva
Dr. John Parrish & Godley-Snell animal facility staff