Novel Signal Transduction Therapeutic Strategies for Paralysis and Pain After Spinal Cord Injury (SCI)

Chen Guang Yu, Ph.D., M.D.
Assistant Professor
Spinal Cord and Brain Injury Research Center
Department of Anatomy and Neurobiology
University of Kentucky College of Medicine
Lexington, KY, USA
OUTLINE
(Therapeutic Strategies)

1 Targeting Individual ERK Isoform and Its Cross-talk with Calpain 1 for Neuroprotection

2 FluBZ Therapy Targeting Multiple Signal Pathways for SCI
Traumatic Spinal Cord Injury (SCI)

- **Permanent locomotor disability**
  - 11,000 new cases occur each year in USA
  - Over 2.5 million SCI patients with paralysis in the world

- **SCI-pain** (a common complication, 70-90%)
  - refractory to conventional analgesic treatment

- **Sustained activation of multiple signal pathways after SCI**
  - *Activation of the ERK1/2 signaling cascade by excitotoxic spinal cord injury.* Yu CG, Yezierski RP. 2005
  - *Calpain in the CNS:* from synaptic function to neurotoxicity.
  - *Cell cycle activation and spinal cord injury.*
    Wu J, Stoica BA, Faden AI. 2011

- **No effective treatments** for Paralysis and Pain after SCI
  - Yu, et al., 2005, 2010; Crown et al., 2006; Zhao et al., 2007, We, et al. 2011
Part 1: Targeting Individual ERK and Its Cross-talk with Calpain 1 for Treating paralysis and SCI-pain


*Role of ERK 1 and 2 in neuronal survival*
Michal Hetman1,2,3 and Agata Gozdz1

**J Pharmacol Exp Ther. (Review) 2006 Dec;319(3):991-7.**

*A death-promoting role for ERK1/2.*
Zhuang S, Schnellmann RG.

**Hypothesis: reducing ERK2, while sparing ERK1, protects against SCI**

ERK2 shRNA sequence

5'-CACCGCACCTCAGCAATGATCATCGAAATGATCATTGCTGAGGTGC-3'

**Lentiviral ERK2 shRNA-GFP vector construction and production**
The role of ERK2 in locomotor function after SCI

Lentiviral-ERK2 shRNA intraspinal injection 1 wk preinjury
Improves locomotor function and tissue sparing following SCI

Spinal ERK2 Knockdown
At 2 wks post-injury in rats

Viral titer:
5X10^7 to 1X10^8

Eriochrome cyanine (EC) stained spinal sections

I: LV-ERK2 shRNA; II: LV-Control

Yu et al., J Neurochemistry 2010
Contributions of ERK1/2 to pain behaviors after excitotoxic SCI

**QUIS:** Quisqualic acid
an AMPA receptor agonist

**ERK1/2 activation after Excitotoxic SCI**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Grooming Behavior</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD98059</td>
<td>4</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6</td>
<td>5</td>
<td>83%</td>
</tr>
</tbody>
</table>

(PD98059 1 µg/15µl delivered to surface of the cord For 1 h pre and immediately post–injection of QUIS)

Self-injurious over-grooming of the affected dermatome

*pERK2, but not pERK1, contributes to the complete Freund’s Adjuvant-induced and formalin-induced pain* (Xu Q, et al, 2008; Alter BJ et al., 2010)
Determination of targets of ERK1/2 activation after SCI

ERK1/2 inhibition (U0126) down-regulates calpain 1 expression 4h after SCI in rats

U0126 iv (2mg/kg) and ip (10mg/kg) pretreatment
LV-Capn1shRNA intraspinal injection 1 wk preinjury improves locomotor function and tissue sparing following moderate SCI in rats

Spinal CAPN1 knockdown
At 2 wks post-injury in rats

Locomotor Function Test

T10
180 kdyn
IH SCI Device
L-E Rats

Viral titer: 5X10^7 to 1X10^8

Eriochrome cyanine (EC) staining

Yu et al., Journal Neurotrauma, 2012

n=10 per group, ANOVA followed by the Bonferroni post hoc test
Determination of CAPN1 targets

Using cocktail antibodies

**Effects of CAPN1 KO on cell signaling pathways after SCI**

<table>
<thead>
<tr>
<th>Cell signaling targets</th>
<th>time points</th>
<th>protein levels (Western blot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-II-Spectrin</td>
<td>24h</td>
<td>no change</td>
</tr>
<tr>
<td>Calpain2</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Caspase 3</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Clevaved caspase 3</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-ERK1/2</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-SHP2</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-AKT</td>
<td>6h, 24h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-p90RSK</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-p53</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>Phospho-p38 MAPK</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>pS6 Ribosomal protein</td>
<td>6h</td>
<td>no change</td>
</tr>
<tr>
<td>NF-kB</td>
<td>24h</td>
<td>no change</td>
</tr>
</tbody>
</table>

CAPN1 deletion increased ERK1/2 activation after acute SCI in mice
CAPN1 deletion exacerbated SCI–pain after acute SCI in mice

CAPN1 deletion showed earlier onset of grooming

CAPN1 deletion worsened SCI–pain after SCI in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Grooming Behavior</th>
<th>Incidence</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPN-KO</td>
<td>5</td>
<td>4</td>
<td>80%</td>
<td>6</td>
</tr>
<tr>
<td>WT</td>
<td>4</td>
<td>3</td>
<td>75%</td>
<td>12.5</td>
</tr>
</tbody>
</table>
ERK1 --/-- mice exhibit exacerbated paralysis in EAE. (Agrawal A, 2006)

ERK1 plays a critical protective role against NMDA injury. (Nakazawa T, 2008)
Part 2: FBZ/FluBZ Therapy Targeting Multiple Signal Pathways for SCI

Fenbendazole (FBZ)

- A benzimidazole anthelminth for animal use only

- FBZ inhibits microtubule formations, and thereby blocking mitosis in nematodes

- FBZ has greater sensitivity for nematodes as compared to mammalian tubulin

- Its influence on the outcomes of ongoing experiments are a concern

Villar et al., 2007; Friedman et al., 1978
FBZ improves locomotor function and tissue sparing after moderate SCI in mice

**Locomotor Function**

- SCI-Control
- SCI-FBZ

- BMS Score
- Days Post-Injury: 1, Pre-Injury, 3, 7, 14, 21, 28, 35, 42

- 35 Days Post-Injury
- 42 Days Post-Injury

**Tissue Sparing**

- Eriochrome cyanine (EC) staining for myelin

**FBZ-medicated feed (8 mg/kg/day) for 4 wks prior to SCI, n=7/group**

Yu CG et al., 2014
FluBZ improved locomotor function and tissue sparing after SCI in rats

- Approved for human use,
- Long term treatment without adverse effects

**Locomotor Function**

<table>
<thead>
<tr>
<th>T10</th>
<th>180 kdyn</th>
</tr>
</thead>
<tbody>
<tr>
<td>IH SCI Device</td>
<td></td>
</tr>
<tr>
<td>SD rats</td>
<td></td>
</tr>
</tbody>
</table>

**Total Tissue Sparing**

FluBZ IP treatment, 3 hrs post-injury for 2 wks (10 mg/kg/day, n=10 per group)

B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice.  
*Ankeny DP, Guan Z, Popovich PG.*

Effects of fenbendazole on the murine humoral immune system.  
*Landin AM, Frasca D, Zaias J, Van der Put E, Riley RL, Altman NH, Blomberg BB.*

FBZ suppresses B cell proliferation and production of antibodies.

FBZ reduced IgG levels and CD45R-positive B cells at lesion site six weeks after SCI in mice

**Immunohistochemistry for IgG**

- Sham
- SCI-Control
- SCI+FBZ

**Immunofluorescence staining for CD45R-B cells**

- SCI-Control
- SCI-FBZ
- Sham

Yu CG et al., 2014
FLuBZ improves recovery of B cell population 4 weeks after SCI

Flow cytometry analysis for CD45RA-positive B cell population
FluBZ inhibited ERK1/2, cyclin B1, and astroglial activation 4 wks after SCI

ERK1/2 Activation

Cyclin B1 upregulation

Astroglial Activation
MAP kinase and pain.
Ji RR\(^1\), Gereau RW 4th, Malcangio M, Strichartz GR.

MAPK activation in nociceptive neurons and pain hypersensitivity.
Obata K\(^1\), Noguchi K.

[Glial cells and chronic pain: from the laboratory to clinical hope].
[Article in French]
Clarke CB\(^1\), Suter MR, Gosselin RD.

Astrocytes—multitaskers in chronic pain.
Hansen RR\(^1\), Malcangio M.
FluBZ attenuates pain behaviors after excitotoxic SCI in rats

Grooming Onset

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grooming Onset (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUIS+Vehicle</td>
<td>11</td>
</tr>
<tr>
<td>QUIS+FluBZ</td>
<td>19</td>
</tr>
</tbody>
</table>

Grooming Area

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grooming Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUIS+Vehicle</td>
<td>2.5</td>
</tr>
<tr>
<td>QUIS+FluBZ</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Grooming Severity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grooming Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUIS+Vehicle</td>
<td>3.0</td>
</tr>
<tr>
<td>QUIS+FluBZ</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Vehicle-treated

- Grooming incidence: 75%
- Onset grooming (days): 11

FluBZ-treated

- Grooming incidence: 40%
- Onset grooming (days): 19
Summary for Part 2
(FBZ/FluBZ therapy)

• Fenbendazole and flubendazole improve experimental outcomes following SCI

• Flubendazole inhibits
  pERK1/2
  cyclin B1
  B-cell response
  astroglial activation

• Flubendazole is clinically approved and could easily be translated to human clinical trials.
Future Direction

**SCI primary injury**

- FluBZ
  - inhibit microtubule formation

**Cell cycle progression at G2/M**

- pERK1/2
  - Cyclin B1-CDK1

**Molecular mechanisms**

- B cells
- Astrocytes
- Microglia

**Cellular mechanisms**

**Pathological mechanisms**

- Autoimmune
- astrogial scar
- Inflammation, Nociception

**Dysfunction**

- Neuronal/axonal damage
- Failed axonal regeneration
- Locomotor deficits
- Chronic Pain
ACKNOWLEDGEMENTS

Dr. James W. Geddes, Ph.D.
University of Kentucky

Drs. Yu/James W. Geddes’s lab
Vimala Bondada
Carolyn Crowdus
Colin Rogers, Ph.D.
Kashif Raza
Sarbani Ghoshal, Ph.D.
Ranjana Singh, Ph.D.
Brantley Graham
Charles Mashburn, Ph.D.
Lauren Thompson
Allie Zeller
Lauren Power
Mackenzie Jones
Jessica Jones

Dr. Robert P. Yezierski, Ph.D.
University of Florida

Dr. Hall’s lab
Dr. Rabchevsky’s lab
Dr. Saatman’s lab
Dr. Springer’s lab
Dr. Sullivan’s lab
Dr. Snow’s lab
Dr. Smith’s lab
Dr. Bondada’s lab
Dr. Gensel’s lab

Ms. Zel Frye,
Ms. Liz Jones,
Ms. Julie Combs
Dr. Jeanie F. Kincer

Funding Sources

• University of Florida Seed Grant:
  “Effects of ERK1/ERK2 siRNA on Spinal Injury Pain”
  PI: Chen Guang Yu, 2004-2005

• Paralysis Project of America Grant:
  “Inhibition of ERK1/2 for treatment for spinal cord injury
  PI: Chen Guang Yu/James Geddes, 2006-2007

• KSCHIRT Grant 7-6A:
  “Inhibition of ERK2 with lentiviral ERK2-shRNA for SCI”
  PI: James Geddes/Chen Guang Yu, 2008-2014

• KSCHIRT Grant 11-19A:
  “Calpain knockdown minimize damage and deficits after SCI”
  PI: Chen Guang Yu/James Geddes, 2012-2015

• NIH CTSA Grant: ULTR000117
  PI: Chen Guang Yu, 2014-2016