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

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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.
  Liu J, Liu MC, Wang KK. 2008

  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

Eur. J. Biochem. (Review) 271, 2050–2055 (2004)

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.

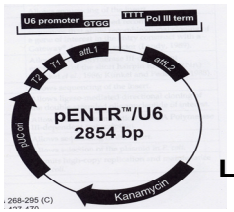
Zhuang S, Schnellmann RG.

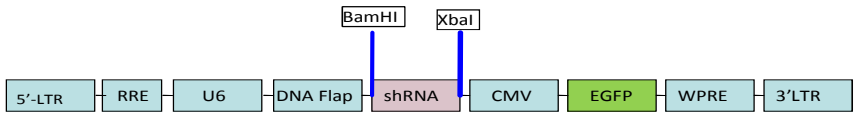
Hypothesis: reducing ERK2, while sparing ERK1, protects against SCI

ERK2 shRNA sequence

sense loop antisense

5'-CACCGCACCTCAGCAATGATCATCGAAATGATCATTGCTGAGGTGC-3'



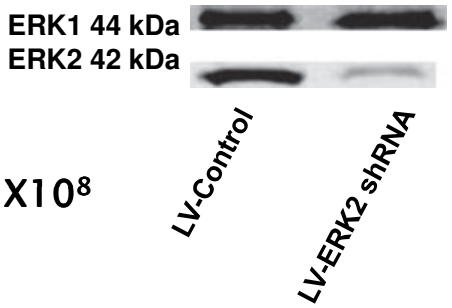


Lentiviral ERK2 shRNA-GFP vector construction and production

The role of ERK2 in locomotor function after SCI

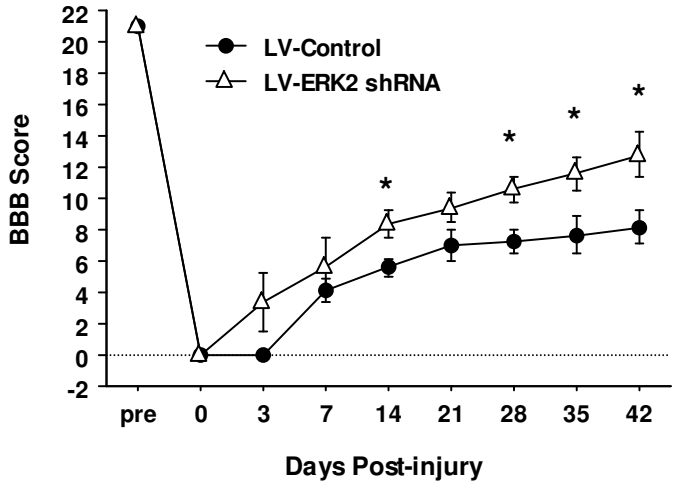
Lentiviral-ERK2 shRNA intraspinal injection 1 wk preinjury improves locomotor function and tissue sparing following SCI

Spinal ERK2 Knockdown At 2 wks post-injury in rats



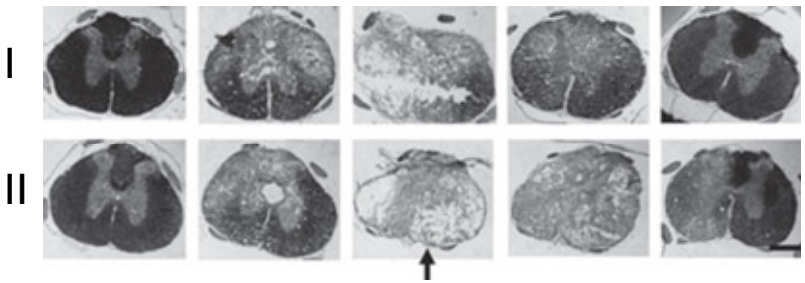
Viral titer:
$5X10^7$ to $1X10^8$

Locomotor function Test



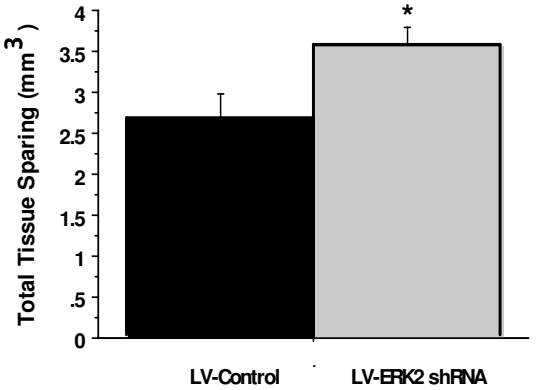
T10
230 kdyn
IH SCI Device
L-E Rats
n=7/group

Eriochrome cyanine (EC) stained spinal sections



6 3 Epicenter 3 6
Rostral Distance from epicenter (mm) Caudal
I: LV-ERK2 shRNA; II: LV-Control

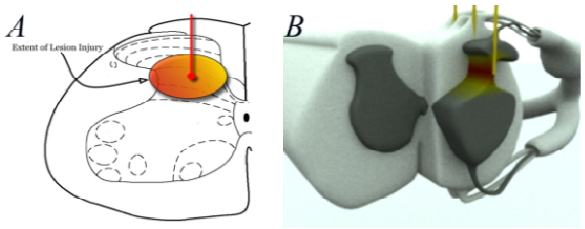
Tissue Sparing Assessment

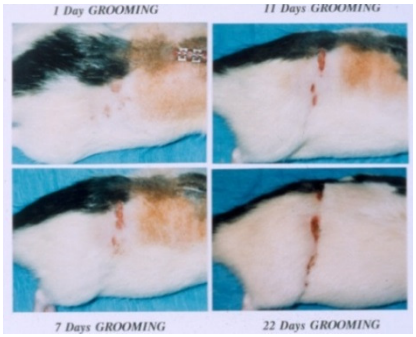


Yu et al., J Neurochemistry 2010

Contributions of ERK1/2 to pain behaviors after excitotoxic SCI

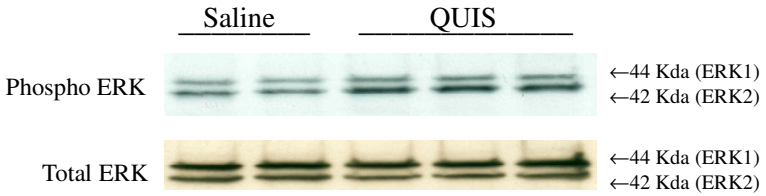
QUIS: Quisqualic acid
an AMPA receptor agonist





Self-injurious over-grooming of the affected dermatome

ERK1/2 activation after Excitotoxic SCI



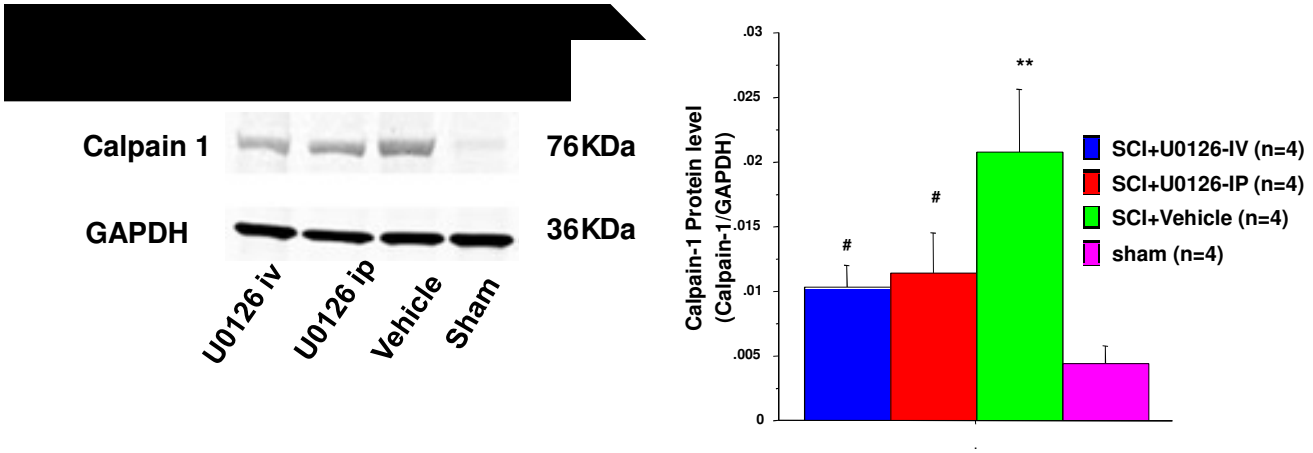
Group	n	Grooming Behavior	Incidence
PD98059	4	0	0%
Vehicle	6	5	83%

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

pERK2, but not pERK1, contributes to the complete Freund's Adjvant-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

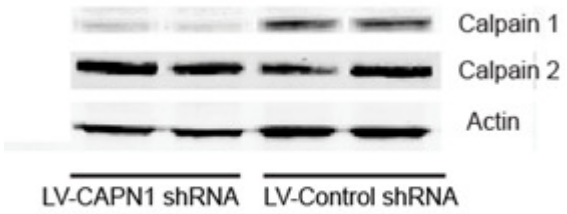


U0126 iv (2mg/kg) and ip (10mg/kg) pretreatment

Role of calpain 1 in SCI

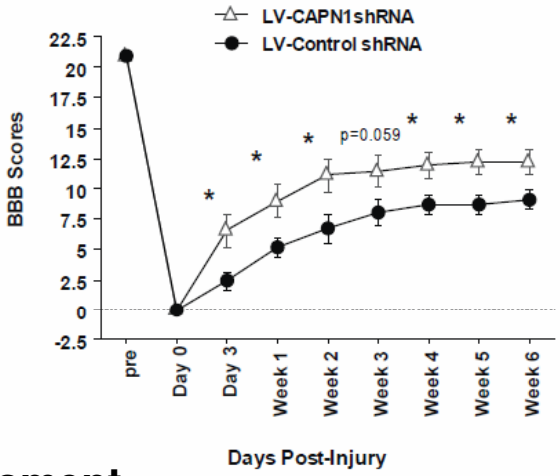
LV-Capn1shRNA intraspinal injection 1 wk preinjury improves locomotor function and tissue sparing following moderate SCI in rats

Spinal CAPN1 knockdown At 2 wks post-injury in rats



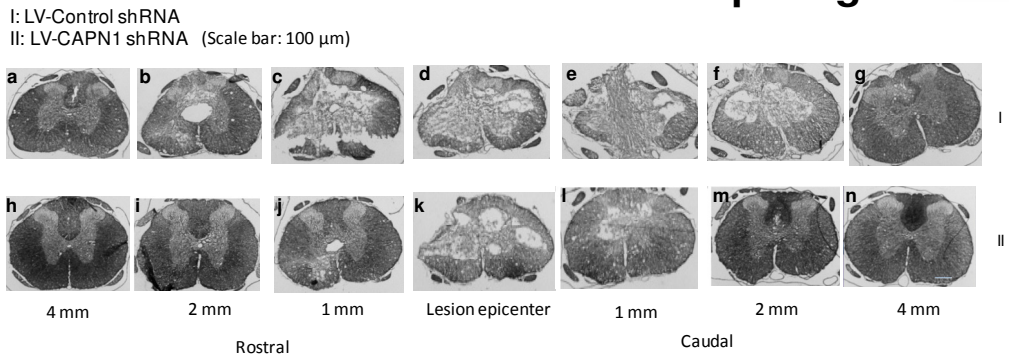
Viral titer:
$5X10^7$ to $1X10^8$

Locomotor Function Test

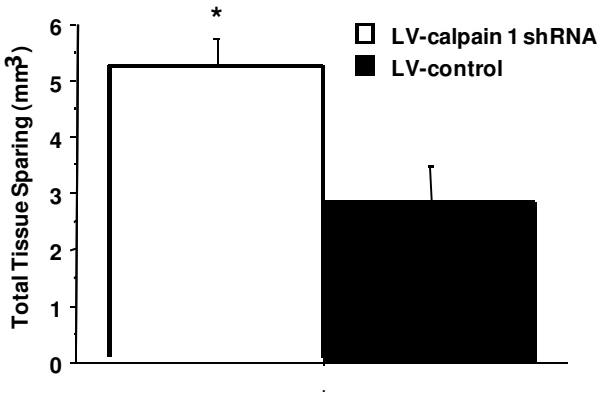


T10
180 kdyn
IH SCI Device
L-E Rats

Tissue Sparing Assessment



Eriochrome cyanine (EC) staining



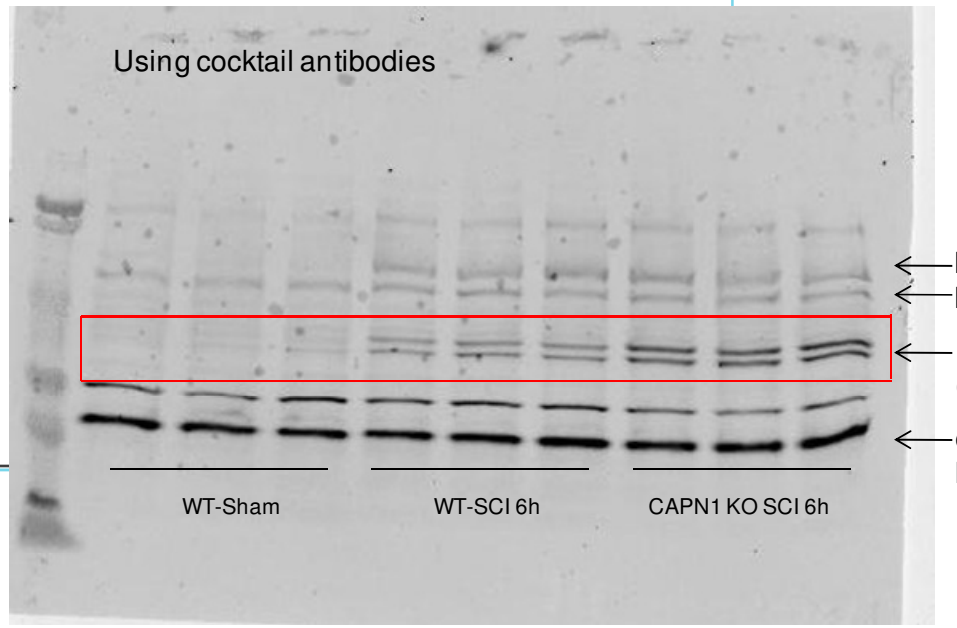
Yu et al., Journal Neurotrauma, 2012

n=10 per group, ANOVA followed by the Bonferroni post hoc test

Determination of CAPN1 targets

Effects of CAPN1 KO on cell signaling pathways after SCI

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

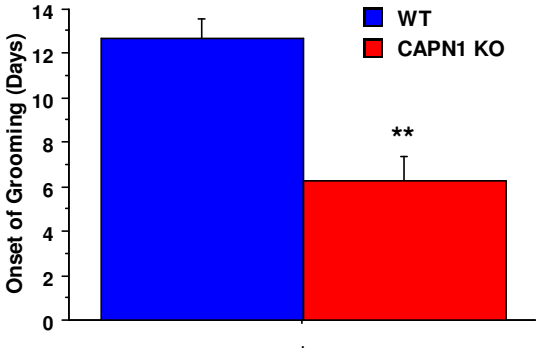


Phospho-SHP2 (72 kDa)
Phospho-AKT (60 kDa)
Phospho-p44/p42 ERK1/2 (44 kDa and 42 kDa)
eIF4E (25 kDa, loading control protein)

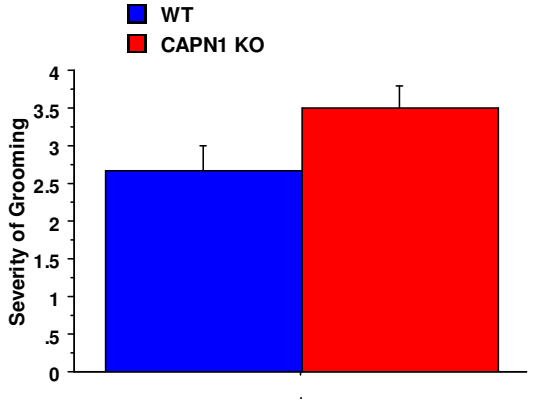
CAPN1 deletion increased ERK1/2 activation after acute SCI in mice

CAPN1 deletion exacerbated SCI-pain after acute SCI in mice

CAPN1 deletion showed earlier onset of grooming



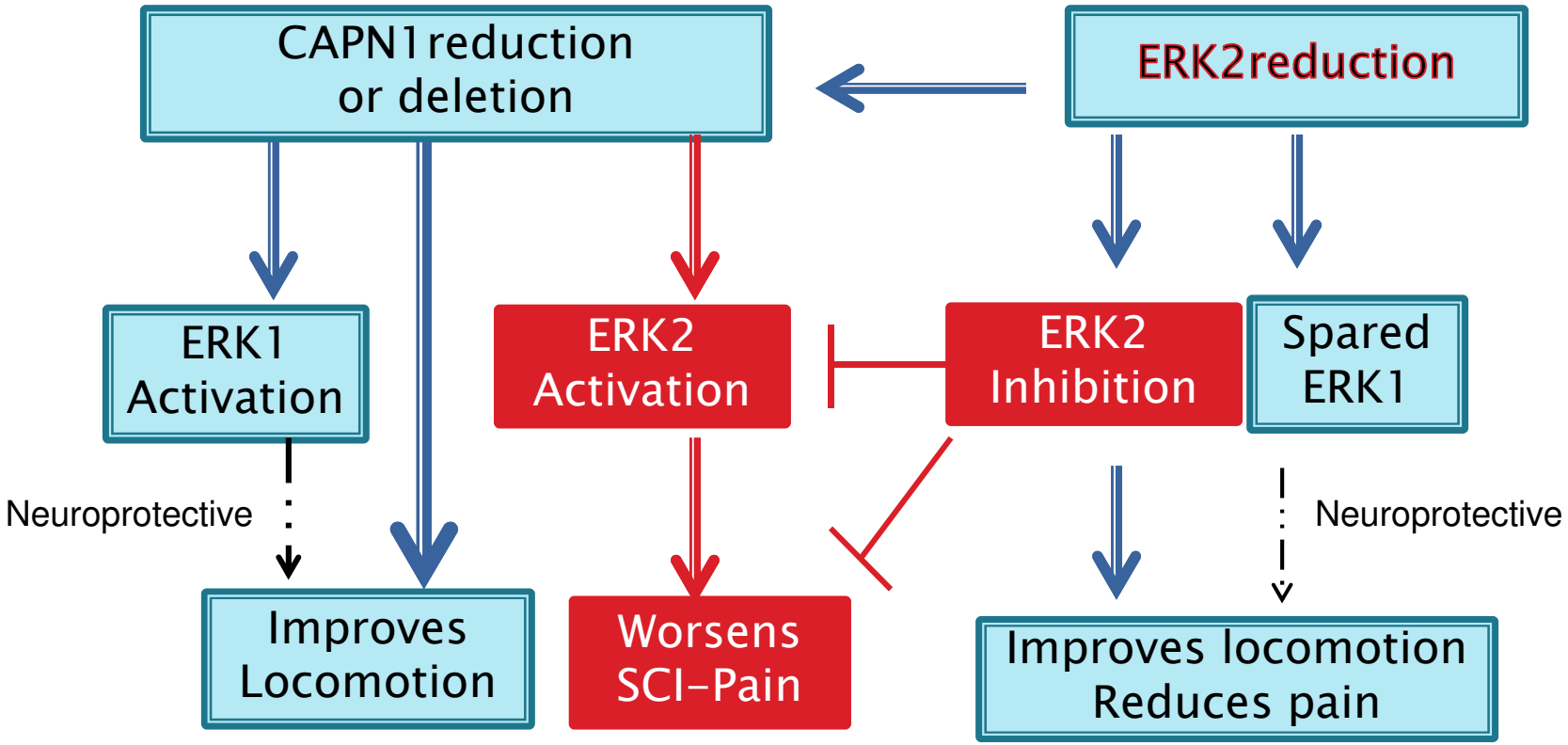
CAPN1 deletion worsened SCI-pain after SCI in mice



CAPN1 deletion exacerbated SCI-pain after SCI in mice

Group	n	Grooming Behavior	Incidence	Onset
CAPN-KO	5	4	80%	6
WT	4	3	75%	12.5

Summary for Part 1: Targeting calpain 1 and ERK2 Cross-talk may provide synergistic effects after SCI



ERK1-/- mice exhibit exacerbated paralysis in EAE. (Agrawal A, 2006)

ERK1 plays a critical protective role against NMDA injury. (Nakazawa T, 2008)

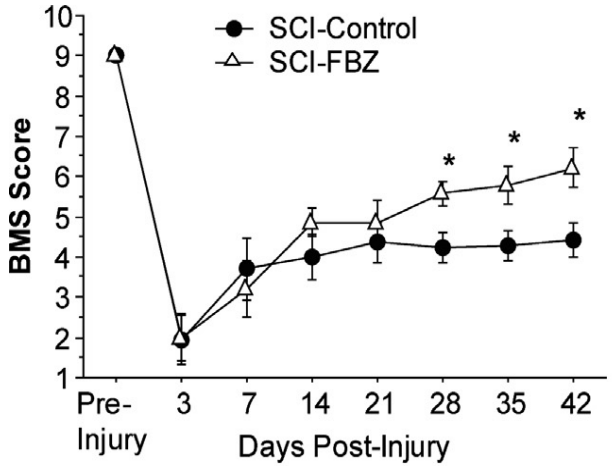
Fenbendazole (FBZ)

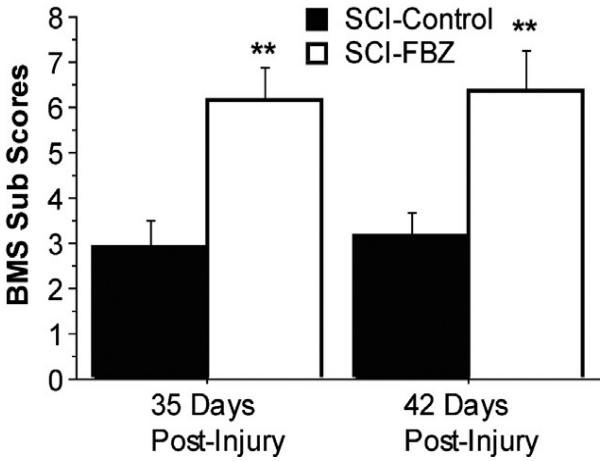
- A benzimidazole anthelminth for animal use only
- FBZ inhibits microtubule formations, and thereby blocking mitosis in nematodes
- FBZ has greater sensitivity for nematodes as compared to mammalian tubulin
- Its influence on the outcomes of ongoing experiments are a concern

Villar et al., 2007; Friedman et al., 1978

FBZ improves locomotor function and tissue sparing after moderate SCI in mice

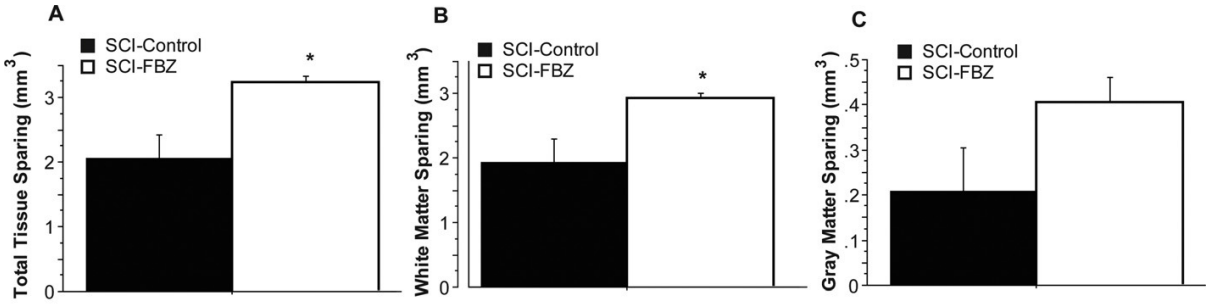
Locomotor Function



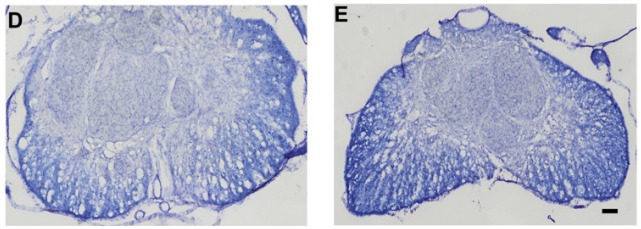


T9
50 kdyn
IH SCI Device
C57BL/6 mice

Tissue Sparing



Eriochrome cyanine (EC) staining for myelin



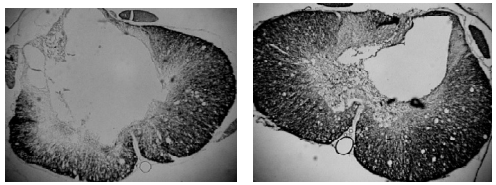
Yu CG et al., 2014

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

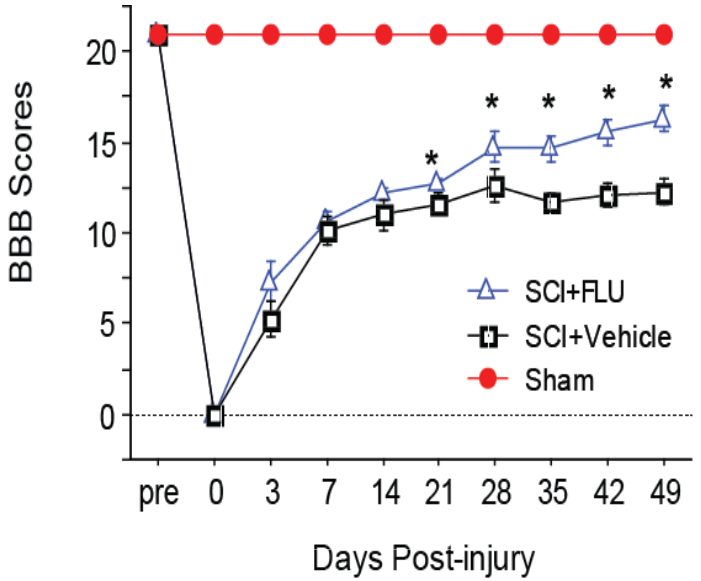
FluBZ improved locomotor function And tissue sparing after SCI in rats

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

Total Tissue Sparing



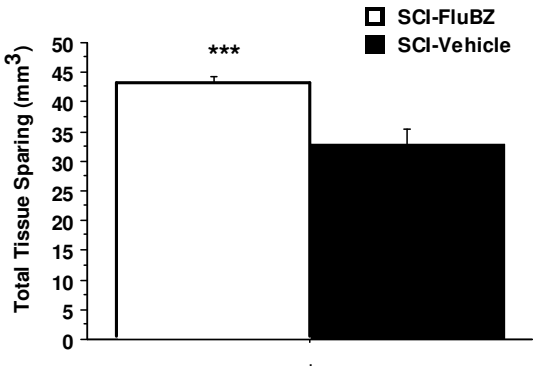
SCI+Vehicle SCIFluBZ+



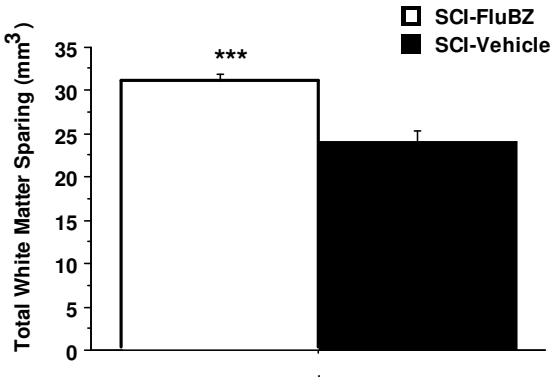
Locomotor Function

T10
180 kdyn
IH SCI Device
SD rats

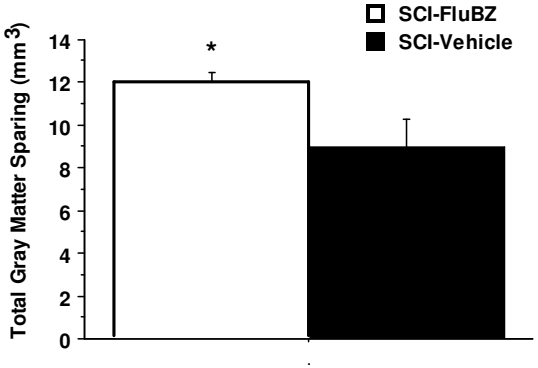
Total Tissue Sparing



White Matter Sparing



Gray Matter Sparing



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

J Clin Invest. 2009 Oct;119(10):2990-9.

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

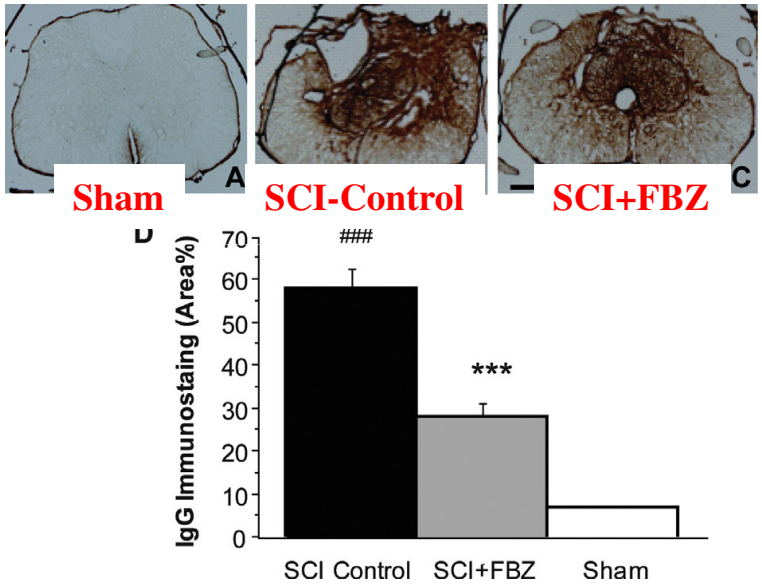
J Am Assoc Lab Anim Sci. 2009 May;48(3):251-7.
Effects of fenbendazole on the murine humoral immune system.
Landin AM, Frasca D, Zaias J, Van der Put E, Riley RL, Altman NH, Blomberg BB.

FBZ suppresses B cell proliferation and production of antibodies.

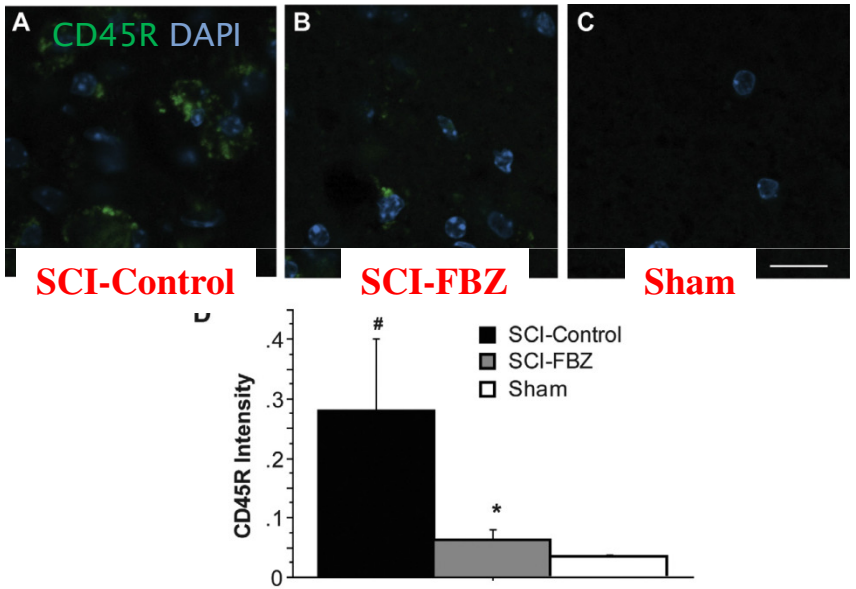
Microtubule inhibitors (Colchicine, vinblastine):
Cell cycle inhibition, antiinflammation, antiproliferation, and inhibition of pERK1/2 (Gaba, 2014, Spagnuolo, 2010)

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

Immunohistochemistry for IgG

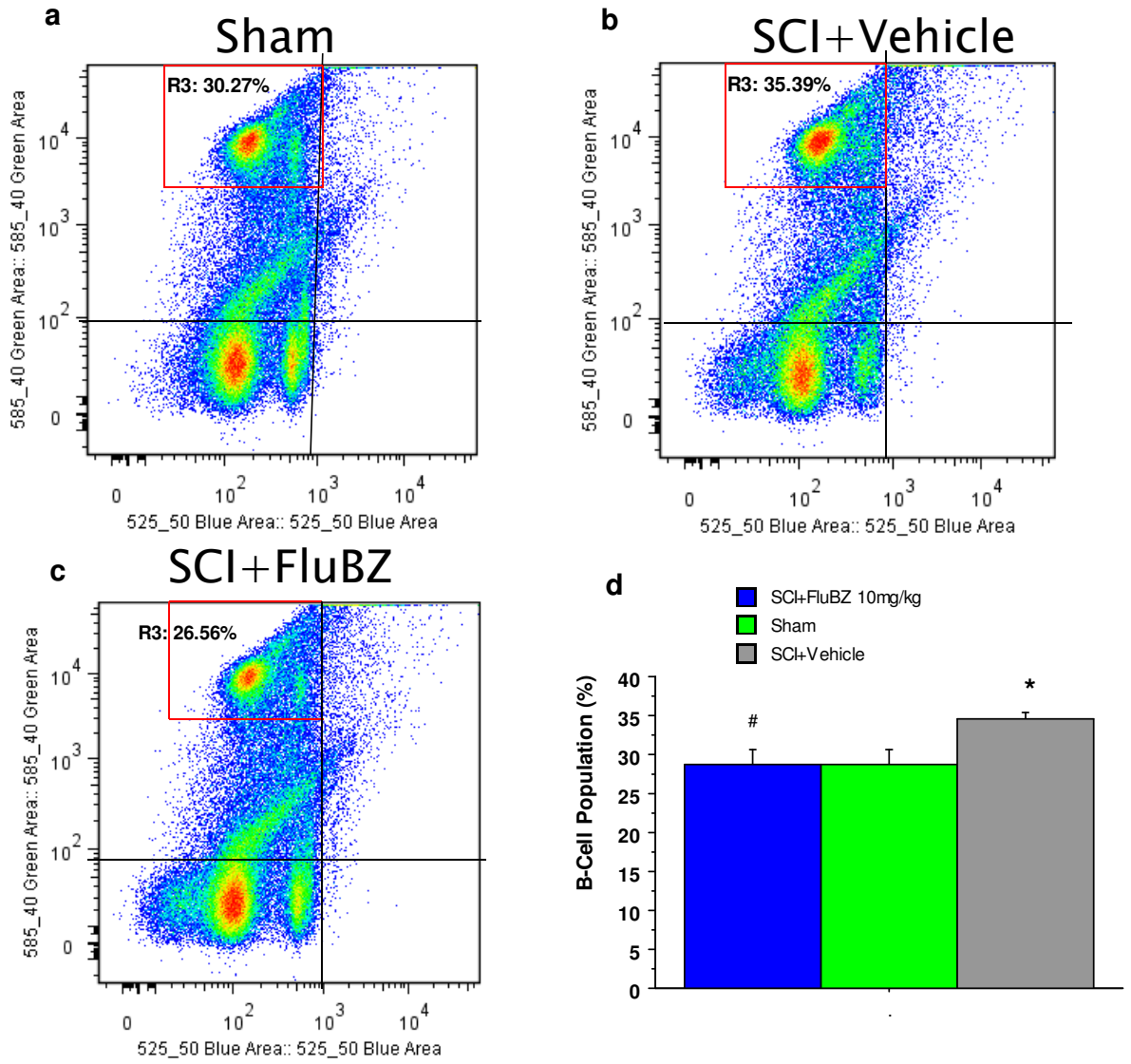


Immunofluorescence staining for CD45R-B cells



Yu CG et al., 2014

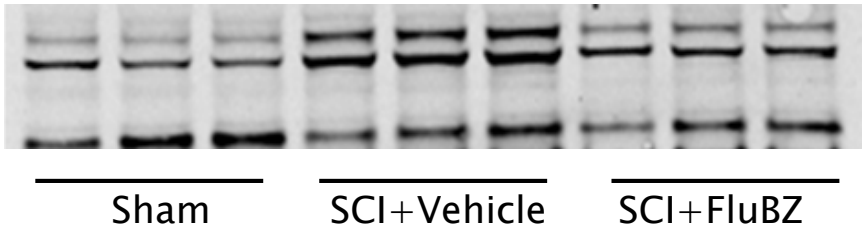
FLuBZ improves recovery of B cell population 4 weeks after SCI



Flow cytometry analysis for CD45RA-positive B cell population

FluBZ inhibited ERK1/2, cyclin B1, and astroglial activation 4 wks after SCI

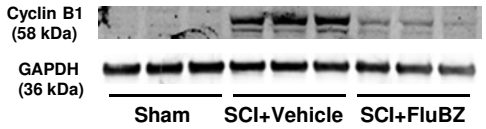
ERK1/2 Activation

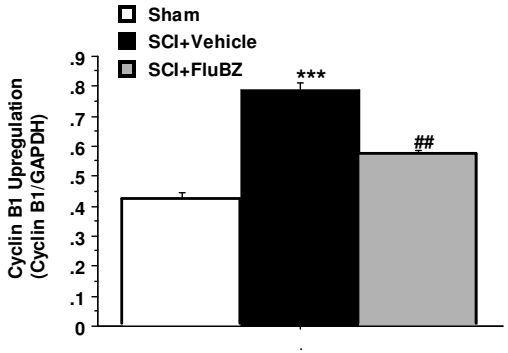


pERK1 44 KDa
pERK2 42 KDa

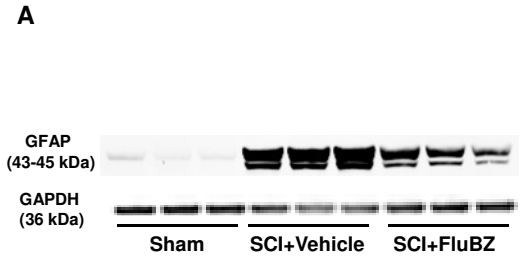
eIF4E (25 KDa)
(loading control)

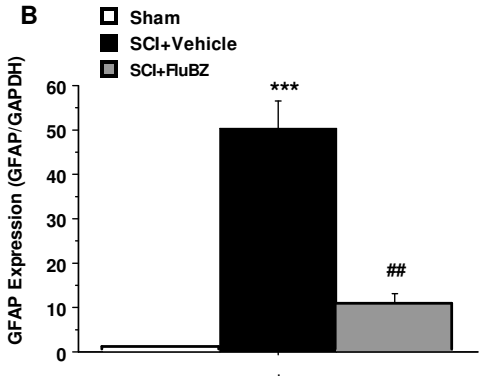
Cyclin B1 upregulation





Astroglial Activation





Brain Res Rev. 2009 Apr;60(1):135-48. doi: 10.1016/j.brainresrev.2008.12.011. Epub 2008 Dec 25.
MAP kinase and pain.
Ji RR[1], Gereau RW 4th, Malcangio M, Strichartz GR.

Life Sci. 2004 Apr 9;74(21):2643-53.
MAPK activation in nociceptive neurons and pain hypersensitivity.
Obata K[1], Noguchi K.

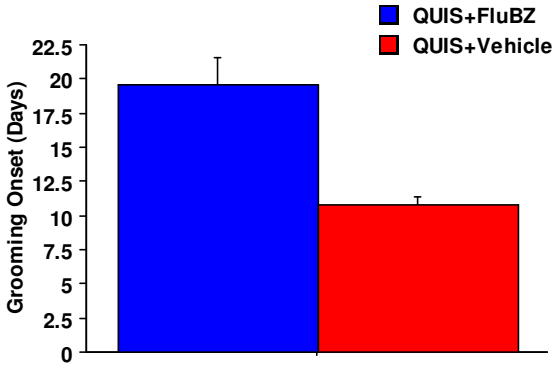
Rev Med Suisse. 2013 Jun 26;9(392):1342-5.
[Glial cells and chronic pain: from the laboratory to clinical hope].
[Article in French]
Clarke CB[1], Suter MR, Gosselin RD.

Eur J Pharmacol. 2013 Sep 15;716(1-3):120-8. doi: 10.1016/j.ejphar.2013.03.023. Epub 2013 Mar 22.
Astrocytes--multitaskers in chronic pain.
Hansen RR[1], Malcangio M.

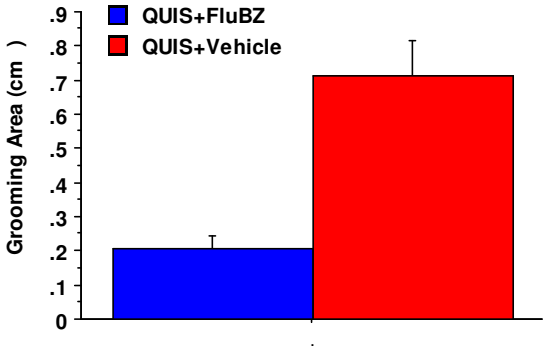
UNIVERSITY OF KENTUCKY

FluBZ attenuates pain behaviors after excitotoxic SCI in rats

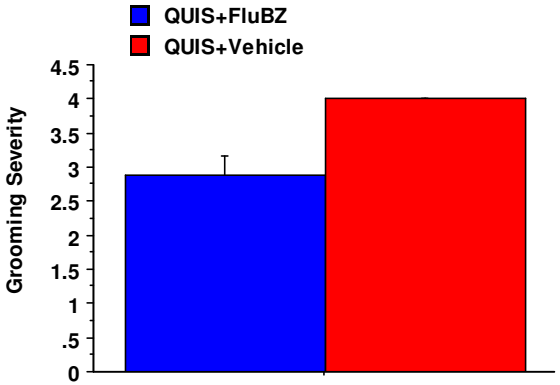
Grooming Onset

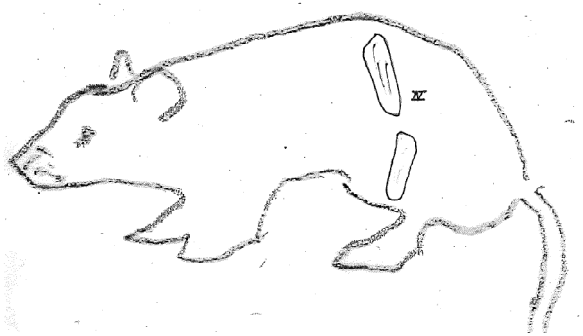


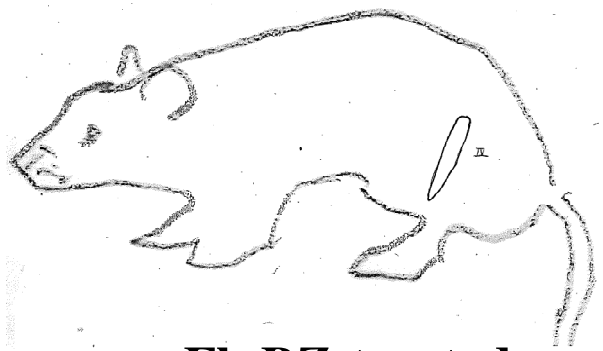
Grooming Area



Grooming Severity





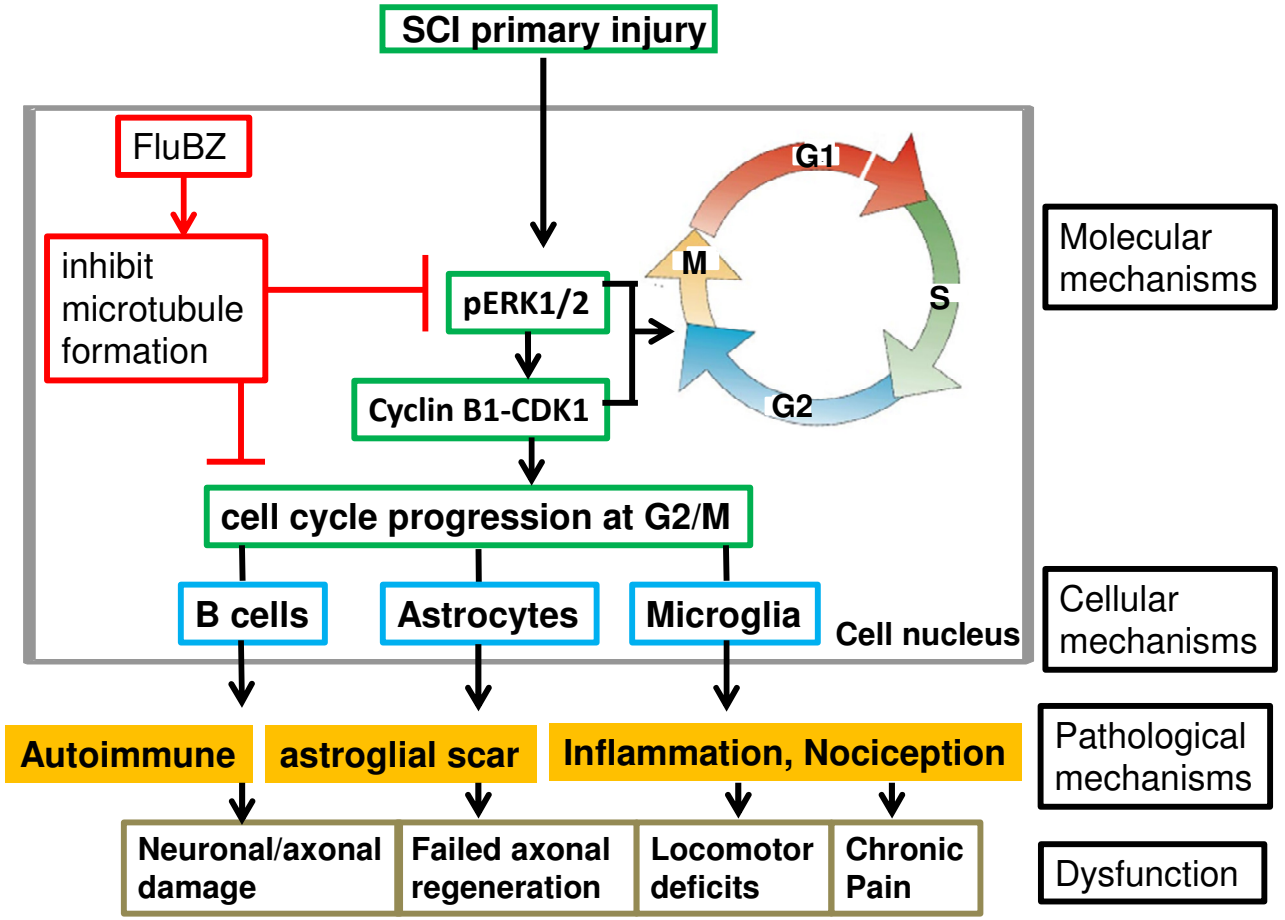


	Vehicle-treated	FluBZ-treated
Grooming incidence	75%	40%
Onset grooming (days)	11	19

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



ACKNOWLEDGEMENTS

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University of Kentucky

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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,
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  "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

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