Potential Therapeutic Effects of Injectable Hydrogel for Controlled Release of Thrombin-Inhibitor Following Spinal Cord Injury

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Disclosures

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Spinal cord injury results in:
- Cavitating lesion
- Lack of permissive substrate
- Insufficient neurotrophic support
- Glial scar barrier

Thrombin after SCI:
- Normally tightly regulated in CNS
- Increased levels in spinal cord after SCI
- Increases neuroinflammation
- Binds PAR receptors resulting in neurodegeneration
- Studies link thrombin to demyelination

**Hypothesis:** Controlled thrombin inhibition will result in reduced glial activation, reduced scar and improved recovery following SCI in rats.
Methods

• An injectable hydrogel was designed to release bivalirudin and provide scaffold support in the injured spinal cord.
• 12 rats were given moderate C4 hemi-contusions using an OSU impactor.
• One week after injury, we performed a re-exposure and injected a hydrogel formulation into the cavity.
• Functional recovery was assessed by successful grasps of a chocolate pellet (FRT) and gait (Catwalk XT).
• Animals were perfused at 3 weeks.
• Histological examination was performed to examine changes to lesion.
The bivalirudin peptide fragment is tagged with a rhodamine fluorescent marker. Here we see the hydrogel and drug dissecting the central canal in an uninjured spinal cord by stereoscopic fluorescence imaging.

Day -7: Behavior (Baseline)
Day 0: Injury
Day 6: Behavior (1 Week Post Injury)
Day 7: Spinal Injection (Treatment)
Day 14: Behavior (1 Week Post Injection)
Day 21: Behavior (2 Weeks Post Injection)
Day 28: Sacrifice

Group 1: Injury + Gel (No Drug) N=4
Group 2: Injury + Gel + Drug N=4
Group 3: Injury + Gel + Drug + hNSCs N=4
Functional assessment demonstrated no significant improvement between treatment groups in gait or forelimb reaching in this pilot study. However, there were differences in gait at two weeks. Extending the assessment beyond 2 weeks and increasing the number of animals may reveal a more significant difference in function.
There appears to be a decrease in GFAP+ cells around the lesion border when treated with hydrogel loaded with bivalirudin. More analysis is necessary to determine the extent of the effect.
Cellular Support

- All animals received a hydrogel injection.
- Large numbers of cells inside of the lesion cavity is rare one month after injury in rats.
- All lesion cavities had approximately 2000 cells/mm², which is a very high density inside of a lesion.

High cell nuclei numbers inside hydrogel filled lesions indicate the hydrogel provides scaffolding support for invading cells to survive inside of the lesion. The identity of these cells remains to be determined, but the hydrogel may provide structural support for neurogenesis.
• **Functional assessment**: We are planning a full scale study with 3 groups to test injury alone, injury + gel and injury + gel + thrombin inhibition. We will perform a more aggressive injury and assess the gait and forelimb reaching for an extra month after injury to discern whether there is a functional benefit.

• **Gliosis**: We are attempting to determine whether there is a morphological change to the astrocytes and whether there are changes to myelin and CSPGs in the area around the lesion.

• **Cellular support**: We are attempting to identify the types of surviving cells in the hydrogel filling the lesion cavity.

• We are assessing lesion volume and cell viability inside of the lesions for the different groups.
Summary

• Uncontrolled thrombin release following CNS trauma is associated with neuroinflammation and reduced recovery.
• Controlled release of bivalirudin from an injectable hydrogel inside an SCI lesion cavity changes the morphology of the glial scar, but we have not yet been able to discern functional improvement.
• Our hydrogel provides scaffolding support to allow cells to survive in high density inside the lesion at least one month after injury.
• More work is needed to identify the changes to the scar and the types of invading cells.