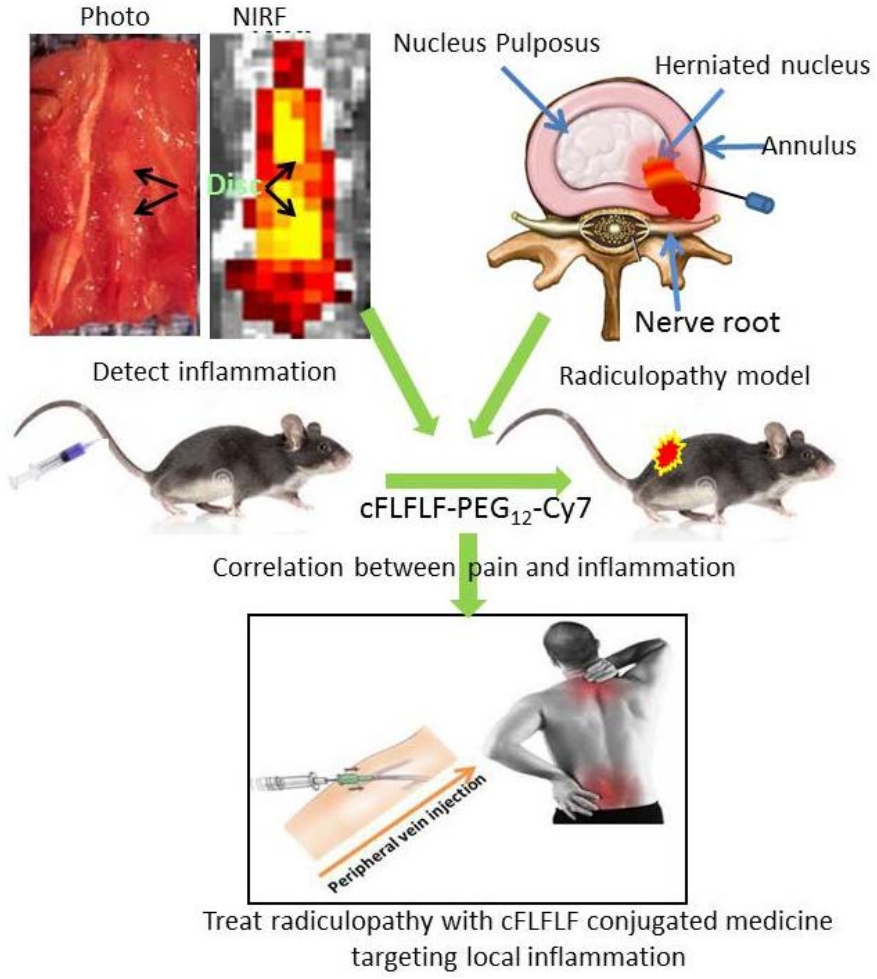
Can we treat radiculopathy via peripheral vein injection?

Joshua Li

Department of Orthopaedic Surgery, University of Virginia

Introduction:

Inflammation plays a key role in the progression of disc degeneration associated radiculopathy. Epidural steroid injection could reduce focal inflammation; however, it is technical demanding. And thus, it is in great need of a targeted therapy through periphery vein injection to suppress focal inflammation evidenced by neutrophil and macrophage infiltration around disc herniation. cFLFLF is a peptide specifically binding to activated neutrophil and macrophage via formyl peptide receptor-1 (FPR1). It has potential to carry therapeutic agent to disc hernia site via peripheral vein injection. To prove the theory, we need test specific binding of cFLFLF and also need a radiculopathy model as lack of a suitable animal model has been a major barrier to advance the understanding of crosstalk between herniated nucleus and nerve root.



Purpose:

The goals of this study are two-folds (Figure 1): 1. To confirm cFLFLF specifically binds to activated neutrophil and macrophage infiltrated around a disc hernia site; 2. Develop a novel mouse model to replicate human radiculopathy. The ultimate goal is to find an effective target therapy for disc herniation associated radiculopathy via peripheral vein injection for human.

Method:

For cFLFLF specific binding, disc degeneration and infiltration of neutrophils and macrophages were detected with Safranin-O and immunostaining after disc injury. FPR1-specific imaging probes cFLFLF-PEG-Cy7 and cFLFLF-PEG-HYNIC-99m16 Tc were administered systemically to sham and disc injury mice. In vivo leukocyte infiltration was tracked by near-infrared fluorescence (NIRF) and single photon emission tomography (SPECT) imaging. The peptide-receptor binding specificity was further investigated with FPR1-/- 1 mouse and in vitro binding assays.

For radiculopathy model, mouse spine was exposed through a transperitoneal approach. L5 and L6 vertebral bodies were identified. For traditional puncture group, L5/6 disc was punctured anteriorly without nerve root exposure. For nerve exposure group, left L5 nerve root was exposed by removing the overlying psoas muscle fibers. For experimental group, in addition to L5 nerve root exposure, L5/6 disc was punctured laterally, where the nucleus protruded toward L5 nerve root. Mechanical hyperalgesia was measured by the electronic von Frey test. The disc herniation and nerve inflammation were confirmed with MRI, Safranin-O staining, as well as in situ immunostaining for inflammatory and neuro peptide markers.

Results:

Massive inflammatory cells were observed in the anterior region of punctured annulus in the injury group. Neutrophils were detected at 1 and 3 days after injury, while macrophages appeared the most at 7 days. Consequently, NIRF and SPECT images revealed specific accumulation of cFLFLF-probes in herniation site of wild type but not in the FPR1-/- mice. Binding specificity of the cFLFLF peptide to FPR1 was confirmed in macrophages isolated from wild type and FPR1-/- mice as well as in Raw 267.4 macrophage cells. Macrophage infiltration was also observed in human degenerated IVD samples.

To further investigate the potential targeted therapy for radiculopathy, we created an acute radiculopathy animal model. In the radiculopathy experimental group, mechanical withdrawal threshold of the left paw was significantly decreased (signifying the increased pain level) compared to the right side that had no nerve exposure

postoperatively up to week 2. The traditional puncture group showed temporally increased pain level on day 2 and the never exposure group showed no difference in pain level. For experimental and traditional puncture groups, MRI showed decreased T2 weighted signals, and histology and immunostaining confirmed disc degeneration and macrophage and neutrophil infiltration around the herniated nucleus. In the experimental group, expressions of IL-1β and IL-6 as well as pain-related markers were increased in the herniated discs and nerve root areas.

Conclusion and Discussion:

For the first time, leukocytes infiltration around acute disc herniation site were targeted and detected directly via cFLFLF binding to FPR-1 expressed on activated neutrophils and macrophages. Such specificity binding of cFLFLF with leukocytes will facilitate future targeted drug delivery to treat acute disc herniation. Additionally, we developed a novel model that replicates the clinical scenario of radiculopathy induced by disc herniation. Using this model, we will evaluate the efficacy of cFLFLF based target therapy for radiculopathy in the near future.

Keywords:

Low back pain, Disc herniation, Leukocyte Infiltration, Formyl Peptide Receptor (FPR)-1, Molecular imaging, Target therapy

Figure 1: Schematic representation of study design via target delivery of therapeutic medicine through peripheral vein.