Investigating the Effects of Nerve Dieback Compounds To Treat Disc-Associated Low Back Pain in a Rat Model

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INTRODUCTION: Low back pain and knee osteoarthritis are the leading causes of disability worldwide [1]. Aberrant nerve ingrowth into intervertebral discs and knee joints is highly correlated with chronic low back pain (LBP) [2, 3] or knee pain [4]. These nerve fibers can be sensitized by mechanical loading and inflammatory factors present in the degenerative environment causing pain [5, 6]. Current OA treatment methods involve opioid or NSAID prescriptions to manage pain, or invasive and expensive surgeries, which can lead to severe side effects [7-9]. An effective long-term treatment that can directly target the aberrant nerve fibers in symptomatic joints is needed to treat pain. **We hypothesize that retraction of nerve fibers in the knee and disc using nerve dieback compounds could alleviate joint-related pain**. Herein, we propose to repurpose vincristine sulfate (Vcr), an anti-cancer drug, and pyridoxine hydrochloride (Pyr), a vitamin B6 supplement, to induce nerve dieback in painful degenerated discs. Vcr, when injected systematically, induces nerve degeneration by depolymerizing microtubules which lead to side effects such as numbness and loss of sensation in cancer patients [10, 11]. Pyr, at high doses and prolonged supplementation systemically, cause burning pain, numbness, tingling and muscle weakness related to nerve degeneration [12]. In this work, we evaluated the safety and efficacy of intradiscal injection of Vcr and Pyr to relieve chronic pain in rat model of disc-associated LBP [13].

METHODS: All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals approved through the University of Nebraska-Lincoln's Institutional Animal Care and Use Committee (IACUC). Vincristine sulfate (Sigma-Aldrich, V8879-5MG) and pyridoxine hydrochloride (Sigma Aldrich, P6280-10G) were dissolved and diluted in 1X PBS to reach the desired final concentrations. In prior work, we performed in vitro screening and identified the optimal dose of Vcr and Pyr to induce nerve dieback of rat dorsal root ganglia neurons with minimal neuronal cell death. To test the **safety** of the nerve dieback compounds, adult female Sprague Dawley rats (Envigo), aged 14 weeks, were acclimated for one week then either 1X PBS (control), 1 mM Pyr, 100 nM Vcr and 500 nM Vcr (n=3 per group) were injected into the L5-L6 disc with a 33G needle. Rat weights and animal distress level (porphyrin staining) were monitored before injection and 10 days after injection. Our lab has also established a rodent model of disc-associated LBP with nerve sprouting in disc correlating to axial hypersensitivity. To test the **treatment efficacy** of nerve dieback compounds, adult female Sprague Dawley rats (Envigo), aged 17 weeks, were randomly divided into sham, injured, Pyr and Vcr groups (n=12 per group), and acclimated to handling and behavioral assays for six weeks before disc injury surgery, where the L5-L6 disc was scraped six times bilaterally with a dissecting needle set to a 3 mm depth [13]. For sham animals, the L5-L6 disc was visualized, then the surgical site was closed in the same manner as the injured animals. On week 11 post-surgery, either 1X PBS, 1 mM Pyr or 500 nM Vcr were injected into the L5-L6 disc of the injured animals. Microcomputed tomography (μCT) images and open arena videos were recorded at baseline, and weeks 10 and 14 to measure disc volume and spontaneous animal behavior [13, 14]. The grip strength assay was conducted every two weeks to assess axial hypersensitivity compared to baseline. Gait changes were recorded every two weeks using high-speed videography as part of the Gait Analysis Instrumentation and Technology Optimized for Rodents (GAITOR) system [15] and analyzed via Automated Gait Analysis Through Hues and Areas (AGATHA) method using MATLAB [16]. At the end of the study, rats were humanely euthanized and L5-L6 motion segments were removed, fixed, decalcified, cryo-embedded, and sectioned for hematoxylin-eosin (H&E). H&E images were scored based on a standardized scoring system to for rat intervertebral disc degeneration [17]. Statistical analysis as stated in the figure captions were performed using GraphPad Prism.

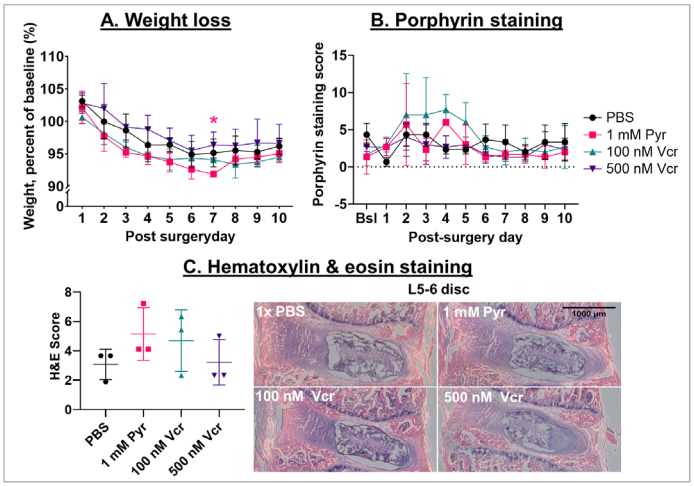

Figure 1: Injection of nerve dieback compounds into adult rat L5-:6 intervertebral disc did not have significant effect on animal weights, stress levels, and disc tissue. (A) Weight loss as a percent of baseline did not significantly differ between groups except on day 7, where 1 mM Pyr had more weight loss than 500 nM Vcr group (p=0.04, Kruskal-Wallis, Dunn's post-hoc). (B) Porphyrin staining, a sign of animal distress, did not change between groups and over time (Kruskal-Wallis). (C) H&E scores were similar between PBS control and treated groups (One-way ANOVA, Tukey's post-hoc), and representative images of H&E staining show signs of a healthy disc.

RESULTS: In the safety study, no significant weight loss (**Fig. 1A**) or behavioral changes in porphyrin staining (**Fig. 1B**) were observed up to 10 days post-injection with 1X PBS, 1 mM Pyr, 100 nM Vcr or 500 nM Vcr. No significant differences between groups were detected in H&E score (**Fig. 1C**). These data suggests that Pyr and Vcr did not alter disc morphology and is safe for intradiscal injection. In the efficacy study, all sham, injured and treated animals significantly decreased distance travelled in open arena across weeks but there were no differences between groups (**Fig 2A**). In the grip strength assay, injured animals had significantly lowered threshold (84% from baseline) compared to sham (96% of baseline) at week 10 post-injury, demonstrating axial hypersensitivity . At 4-week post-intradiscal injection (week 15), 1 mM Pyr and 500 nM Vcr treatment groups exhibited increased thresholds by 5.8% and 3.5% compared to injured PBS-control group; however, these differences did not rise to the level of significance (**Fig. 2B**).

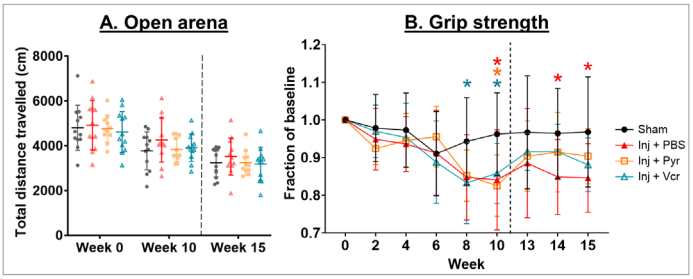

Figure 2: Nerve dieback compounds injected intradiscally into injured discs did not change animal's open arena behavior and did not induce hypersensitivity. (A) Total distance travelled in open arena were not significantly different between groups (2-way ANOVA, Tukey's post-hoc). (B) Grip strength thresholds, as a fraction of baseline, decreased after disc injury and all injured groups reached significance compared to sham on week 10. After Pyr and Vcr intervention on week 11 (dashed line), thresholds of treated animals trended upwards until week 15 but was not significantly different to either sham or injured groups (2-way ANOVA, Tukey's post-hoc).

DISCUSSION: Results suggest that Pyr and Vcr are safe for local intradiscal injection and did not induce disc degeneration or hypersensitivity within 4 weeks post-injection. Immunostaining to label nerves in the L5-L6 discs will be performed to assess nerve dieback in discs.

SIGNIFICANCE: The findings of this research will advance the development of therapeutics specifically related to joints with aberrant nerve growth and impact the quality of life of chronic disc-associated LBP and knee OA patients. This work will also further the scientific understanding behind mechanisms of nerve dieback in relation to pain.

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