Impact of Chronic Ethanol Intake on Intervertebral Disc Degeneration and Pain in (C57BL6) Mice

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Abstract Introduction: Low back pain (LBP) is a leading cause of disability worldwide and ranks among the top conditions contributing to Years Lived with Disability. Intervertebral disc (IVD) degeneration is a major pathological driver of chronic LBP, leading to progressive structural and biochemical changes within the disc. Chronic alcohol consumption has been linked to heightened nociceptive sensitivity and is a risk factor for chronic pain conditions. However, the impact on disc health and degeneration remains poorly understood. SPARC (secreted protein acidic and rich in cysteine) is a matricellular protein that is involved in maintaining the extracellular matrix (ECM). SPARC-null mice represent a genetic model of progressive, age-dependent IVD degeneration characterized by axial discomfort and cold hypersensitivity resembling discogenic pain in humans. We hypothesized that chronic ethanol intake exacerbates IVD pathology and pain-like behaviors in this mouse model.

Methods: Male and female SPARC-null mice and wild-type mice (7–10 per group, 6–8 months old) were used. Mice were divided into two treatment groups receiving either 20% (v/v) ethanol or water under the Intermittent Access Two-Bottle Choice (IA2BC) paradigm, where mice had 24-hour access to 20% ethanol and water on Monday, Wednesday, and Friday, all other days they had two bottles of water. Control mice received two water bottles throughout the entire experiment. This intermittent schedule creates alternating periods of drinking and abstinence, promoting escalation of intake over time. Ethanol exposure ranged from 9 to 14 weeks and was conducted in 3 cohorts. Ethanol and water consumption were recorded to calculate intake in grams of ethanol per kilogram of body weight per 24 hours (g/kg/24 h) and ethanol preference in percentage of ethanol consumption over total fluid consumption (%). Behavioral assays assessing pain and affective function including von Frey, acetone and hot plate tests for radiating leg pain, grip strength and tail suspension assay for axial discomfort, black box for weight-bearing, open field for voluntary activity and anxiety, and the sucrose splash test for depression. Behavioral data were analyzed using three-way ANOVA (sex × strain × treatment) with Šídák post hoc corrections and $p < 0.05$ was considered statistically significant. For the pilot bulk RNA-sequencing analysis, the 5 lumbar intervertebral discs were collected from each animal and processed for RNA extraction. Each sample yielded >1 ng of RNA after DNase treatment, with a minimum RNA integrity number (RIN) of 5.8, as assessed by Agilent BioAnalyzer 2100. Differential and Pathway analysis were conducted by RStudio using edgeR clusterProfiler ($p < 0.05$, $|\log_2 FC| > 1$). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Results: Drinking behavior differed between males and females, with females showing higher ethanol intake when normalized to body weight in both strains. 14 weeks of ethanol exposure produced a significant main effect on grip strength ($p < 0.05$) in both sexes, indicating increased axial discomfort, and a main effect on cold sensitivity in the acetone test ($p < 0.05$). Other behavioral assays, including mechanical (von Frey), thermal (hot plate), axial discomfort (tail suspension), sucrose splash, open field, and weight-bearing, revealed evidence of treatment, strain or sex-dependent modulation of pain-related behaviors under ethanol exposure. Exploratory bulk RNA sequencing was performed on lumbar intervertebral disc (IVD) tissue obtained from male wild-type C57BL/6J sham-operated mice exposed to alcohol (N=4) or water (N=5) for nine weeks. Differential expression analysis identified transcriptional changes in genes and biological pathways related to inflammation, extracellular matrix remodeling, and sensory innervation.

Discussion: These findings suggest that chronic ethanol consumption interacted with degenerative disc pathology to exacerbate pain-related behaviors and IVD pathology. Ongoing and future transcriptomics and methylation analyses aim to correlate behavioral outcomes with molecular changes in IVDs, spinal cord, and blood, with additional studies needed to define causal mechanisms linking ethanol exposure to discogenic pain.

Significance / Clinical Relevance: This study provides novel evidence linking chronic alcohol consumption to the exacerbation of intervertebral disc degeneration and discogenic pain, suggesting potential lifestyle-related contributions to chronic LBP risk.