Pioglitazone Treatment Decreases the Effects of Chronic Alcohol Consumption on Intervertebral Disc Degeneration

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INTRODUCTION: In the U.S., approximately 15% of young adults between ages 18-25 have alcohol use disorder[1] (AUD) and nearly 10% of youths ages 12-20 reported binge drinking within the past month[1]. Heavy alcohol use is known to cause a variety of health conditions, including many that are implicated in back pain, such as spondyloarthritis, osteoporosis, and diabetes[2,3]. One major contributor to back pain is intervertebral disc (IVD) degeneration. However, while several non-scientific studies report a correlation between painful IVD degeneration and alcohol consumption, to our knowledge, no scientific publications exist that study the effect of alcohol consumption on IVD health. Pioglitazone (PIO), a PPAR-γ agonist and antidiabetic drug, has been shown to protect against alcohol-induced expression of pro-inflammatory cytokines in neurons[4]. In IVDs, oral PIO administration decreased pro-inflammatory responses and matrix degradation while promoting matrix production in degenerated nucleus pulposus cells and tissue[5]. The aim of our study was to investigate if chronic alcohol consumption is associated with IVD pathologic changes and to assess if oral PIO treatment could mitigate such detrimental effects.

METHODS: Mouse experiments were performed with IACUC approval. Adolescent (8-10 weeks-old) female and male C57BL/6J mice were randomly divided into four groups: control (CTR; n=5/sex), ethanol (EtOH; n=5 male, n=4 female), control + PIO (CTR+PIO; n=5/sex), and ethanol + PIO (EtOH+PIO; n=5/sex). To achieve a constant blood alcohol volume of 0.12%, EtOH and EtOH+PIO mice were acclimated to ethanol in their drinking water over a period of 2 weeks, starting at 0% w/v ethanol and increasing 5% w/v every 3-4 days until reaching 20% w/v ethanol, which then was maintained for 10 weeks. Starting at week 10, EtOH and CTR mice were treated daily with either 100 µL PIO (10 mg/kg/day in 100 µL methylcellulose vehicle) or 100 µL vehicle alone via oral gavage for 7 days. Mice were euthanized, and L3-5 IVD motion-segments were collected. IVDs were fixed in formalin, decalcified, paraffin embedded, and sectioned for histology (Safranin O/Fast Green (SafO) and hematoxylin & eosin (H&E)). Two IVDs/mouse were assessed for IVD morphology, glycosaminoglycan (GAG) content, cellularity, and notochordal band area. IVD degeneration was assessed using a modified Tam IVD grading system. Data were analyzed using ImageJ. One-way ANOVA (GraphPad Prism 10) with subsequent post hoc testing determined significance $p<0.05$; data is displayed as median with range.

RESULTS: EtOH mice weighed significantly less than CTR mice, regardless of PIO treatment (female: EtOH: 25±10% $p=0.036$, EtOH+PIO: 29±7%, $p=0.01$: male: EtOH: 34±6% $p=0.008$, EtOH+PIO: 32±9% $p=0.011$) and, independent of treatment or sex, drank ~50% less water. Histological evaluation of IVD degeneration suggested that EtOH consumption caused degenerative changes in the extracellular matrix (ECM). Degenerative changes were most pronounced in the nucleus pulposus (NP), occurring as granulated structures often accompanied by fissures. The notochordal (NC) band in EtOH mice appeared compressed, and the cells appeared smaller and less vacuolated (Figures 1+2A). PIO treatment mitigated these degenerative changes in both sexes (Figure 1A+B). As suggested by the flattened NC band, the NC area was significantly decreased in female EtOH mice, which did not reach significance in males (Figure 2). The compressed band might have caused a relative increase in cellularity, as suggested by the significantly increased NC band cellularity in male IVDs. No changes were observed in female IVDs. Assessment of the GAG-rich NP indicated that chronic alcohol consumption caused a decrease in GAG in the NP of male EtOH mice, which was restored after PIO treatment. In contrast, in female IVDs, the GAG content was more variable within groups and remained at high levels after chronic EtOH ingestion.

DISCUSSION: Chronic alcohol consumption is known to be detrimental to biochemical cartilage composition and morphology[6], but not much is known about its effect on IVD health. Our results in adolescent mouse IVDs indicated a deleterious effect of chronic alcohol consumption on IVD health, with a notable impact on NP structure. While the effects of ethanol on notochordal cells differed between female and male mice, treatment with the PPAR-γ agonist PIO partially reversed the detrimental effects of alcohol on IVD morphology in both sexes. PIO has previously been shown to promote ECM production and reduce pro-inflammatory cytokines in human NP cells[5]. In other tissues, PIO treatment protected against alcohol-induced neuronal damage[4] and alveolar macrophage dysfunction[7], indicating that PIO is a safe and potent drug for IVD degeneration treatment. The focus of this study was to histologically assess the potential of PIO to halt or reverse alcohol-induced degenerative IVD changes. Further examination will include investigations of the effects of alcohol and PIO treatment on IVD pro-inflammatory responses and matrix metabolism using molecular assays.

CLINICAL RELEVANCE: This study addresses the under-investigated relationship between chronic alcohol consumption and intervertebral disc degeneration. The presented results suggest PIO as a safe, effective, and non-operative intervention to treat IVD degeneration to improve the quality of life of discogenic back pain patients, especially those people with AUD. The observed sex-dependent effects of EtOH emphasize the need for including both sexes in pre-clinical and clinical studies.

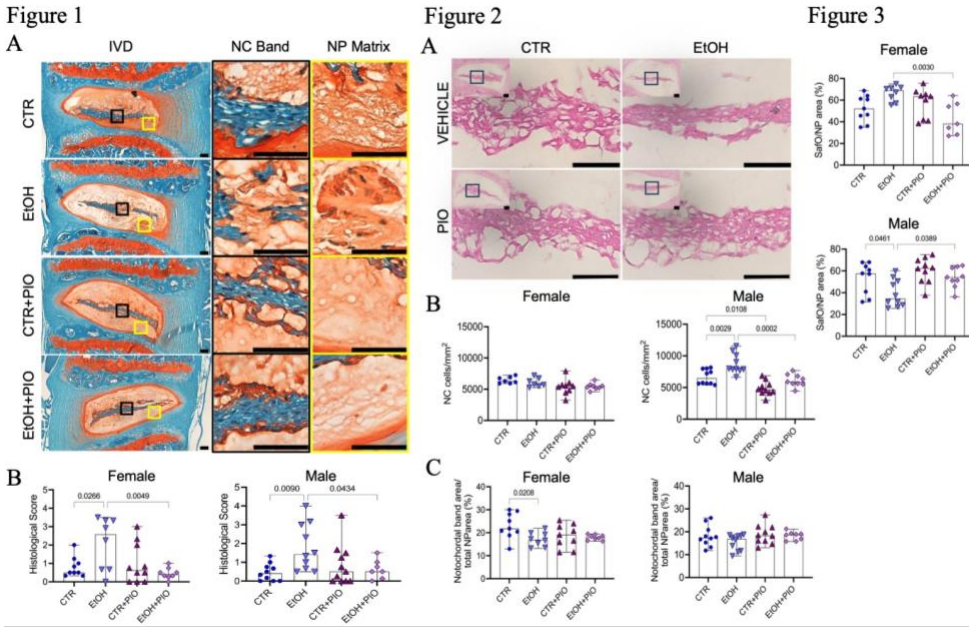

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Figure 1. Chronic EtOH consumption induced IVD degeneration which was mitigated after PIO treatment. Representative images of mouse IVDs. Chronic EtOH consumption caused **A)** altered NC band morphology (black ROI) and granulated structures (yellow ROI) in the NP of EtOH mice. **B)** Quantification of female and male IVD scoring Red: GAG, Blue: Collagen; scalebar: 50um, n=1-10

Figure 2. Chronic EtOH consumption affected NC band cellularity and area. A) The NC band in EtOH mice appeared compact and flattened with less highly vacuolated cells. This observation was supported by the **B)** decreased NC area in female mice but did not reach significance in males, and **C)** cellularity in the NC band increased with chronic EtOH consumption in males but did not alter cellularity in female NC bands. Pink = cells, Purple = nuclei; scale bar: 50um, n=1-10.

Figure 3. GAG quantification in the NP suggested decreased GAG content in male EtOH mice, which was reversed after PIO treatment. In contrast, GAG content was not affected by EtOH in female mice.

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