TACSM Abstract

Alcohol-mediated Changes in Myoblast Hif-1α Protein Expression and Relationships with Decreased Differentiation Capacity: A Preliminary Analysis

COLBY C. NEWMAN[1], ALEXANDRA L. KHARTABIL[1], MATTHEW R. DILEO[1], ZENAIDA A. VILLALBA[1], DANIELLE E. LEVITT[1]

[1]Metabolic Health & Muscle Physiology Laboratory, Department of Kinesiology, Texas Tech University, Lubbock, TX

Category: Masters

Advisor / Mentor: Levitt, Danielle (danielle.levitt@ttu.edu)

ABSTRACT

Alcohol-mediated myopathy, or decreased functional skeletal muscle (SKM) mass, is highly prevalent among individuals with alcohol use disorder (AUD). SKM regeneration aids in maintaining functional SKM muscle mass. Using an in vitro (myoblast) model, we showed that alcohol (EtOH) treatment decreases differentiation capacity in association with decreased glycolytic function, but the underlying mechanisms are incompletely understood. Hypoxia-inducible factor (Hif)-1α is a transcription factor that promotes glycolysis, the bioenergetic pathway that primarily supports myoblast energetic demands. EtOH can increase or decrease Hif-1α expression in various cell types, but this has not been assessed in myoblasts. **PURPOSE:** Test the hypothesis that alcohol decreases HIF-1α expression in myoblasts in association with decreased differentiation capacity. **METHODS:** C2C12 myoblasts (N=5 biological replicates) were proliferated (3 days; D0) and differentiated (5 days; D5) with 0 or 50 mM ethanol (EtOH). At D0 and D5, a portion of cells were harvested for protein isolation and another portion were fixed for assessment of differentiation indices. After protein was isolated from cells, western blotting and enhanced chemiluminescence were used to detect Hif-1α and β-actin contents, and Hif-1a expression was quantified and normalized to β-actin. HEMA 3 stains were applied to fixed cells and images were captured using bright-field microscopy. Differentiation indices (fusion index, myotubes per field, average nuclei per myotube, and total nuclei) were quantified for 16 images from each sample using ImageJ. Differences between conditions (0 and 50 mM EtOH) in Hif-1a expression and differentiation indices were analyzed for each time point (D0 and D5) using paired samples t-tests or Wilcoxon signed rank tests. Delta scores (50 mM-0 mM) were calculated for each outcome at each time point, and Spearman correlations were run between delta scores for Hif-1a and each differentiation index. **RESULTS:** EtOH treatment did not significantly alter Hif-1a expression, although a moderate effect size (g=0.579) for EtOH to increase Hif-1a expression at D5 suggests that a larger sample size would reveal this effect. EtOH decreased fusion index at D0 ($p=.01$) and D5 ($p<0.01$), myotubes per field at D0 ($p=0.03$) and D5 ($p<0.01$), and total nuclei at D0 ($p=.04$). Correlations indicate significant ($p<0.01$), negative associations between delta scores for Hif-1a expression with delta scores for fusion index at D0 and D5 and myotubes per field at D0. **CONCLUSION:** We did not detect an EtOH-induced alteration in Hif-1a expression, but effect sizes and relationships between delta scores suggest that 50 mM EtOH may increase Hif-1a in association with decreased differentiation, possibly as a compensatory response. Additional experiments will verify the impacts of EtOH on Hif-1a expression in myoblasts and assess additional parameters that may reveal a mechanism by which EtOH decreases myoblast glycolytic function and differentiation capacity.