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"The Effects of Mitochondrial Catalase Overexpression on Alcohol-Induced Skeletal Toxicity"

Alcohol induces deleterious effects on the quantity and quality of bone in the skeleton. The mechanism of these effects is believed to be through increased concentrations of reactive oxygen species (ROS) within bone cells. Hydrogen peroxide (H_2O_2) is a form of ROS that is converted to water (H_2O) and oxygen (O_2) by the enzyme catalase. Our previous studies have demonstrated that nicotinamide adenine dinucleotide phosphate oxidase (NOX) enzymes 2 and 4 are contributors of ROS within the cellular environment of bone; however, selective knockout of these enzymes did not lead to complete protection against alcohol's inhibition of osteoblastogenesis and upregulation of osteoclastogenesis. This led to speculation that mitochondrial derived ROS also contributes to increased dysregulation of bone in ethanol (EtOH) exposure, due to the elimination of alcohol's effects with addition of the glutathione precursor N-acetylcysteine (NAC). Also, recent studies demonstrated mitochondrial catalase (mCAT) overexpression was protective against skeletal senescence mediated by H_2O_2. We subjected wildtype and mCAT overexpressed mice to a four-day binge ethanol model previously shown to replicate the chronic negative effects of alcohol. Genotypes were confirmed by collecting tail tips. QRT-PCR analysis of femur shaft and marrow RNA was used to determine the concentrations of osteoblast, osteoclast, and adipogenic markers within the bone. Procollagen 1a1 concentration was determined by running an ELISA assay on serum. Gene expression of osteoblast markers Col1a1 ($p=.008$) and Smpd3 ($p<.001$) was down regulated following ethanol exposure in both wildtype and mCAT mice. Osteoclast differentiation markers Calcr and RANKL showed differing results. Calcr ($p=0.008$) was upregulated in mice shafts exposed to EtOH binge while RANKL was down regulated in the shaft ($p=0.016$) but upregulated in the bone marrow of the mCAT mice exposed to the EtOH binge ($p=0.058$). Within the four-day binge model, there was no statistically significant change in the expression of adipogenic markers Fabp2, Ppar-γ, and Ebf-1 within the bone marrow. Also, the characteristic increased weight loss in the mice exposed to the ethanol treatment held true for both wildtype and mCAT mice ($p<.001$). No statistical change in serum concentration of procollagen 1a1 in the mCAT genotype was observed in the ELISA, suggesting protection from EtOH's effects. Our data demonstrates overexpression of mitochondrial catalase does not protect against alcohol's effects on gene expression in the femur shaft and marrow, but that H_2O_2 signaling plays a role in the systemic concentration of procollagen 1a1.

Special Instructions: The abstract is a summary of the project. Do not to exceed one page. Do not change margins, font style or font sizes on this page. **Use this format only- do not modify!!!**