Introduction

Alcohol-associated liver disease (ALD) is one of the most prevalent liver diseases in the United States. Approximately 140,000 people die from alcohol-related causes annually, making it one of the top five causes of death in the country. ALD is characterized by three major stages, progressing from steatosis to steatohepatitis/fibrosis, to finally cirrhosis, an end-stage liver disease. Unfortunately, there are no effective treatments for ALD besides abstinence, or in extreme cases, liver transplant. Cofactors are used for the treatment of inflammation, but long-term use is associated with adverse side effects and risk of infection. Alcohol consumption has extensive negative effects on bodily processes. Increased ethanol (EtOH) metabolism results in the dysregulation of energy signaling and lipid metabolic pathways through an elevation in reactive oxygen species (ROS) and acetaldehyde. Additionally, chronic alcohol intake alters mitochondrial function via impairment of oxidative phosphorylation leading to decreased oxidative metabolism, ATP synthesis, and hepatoocyte tolerance to stress. Mitochondrial proliferation and increased activity of critical enzymes involved in mitophagy can regulate healthy mitochondria function. Dihydromyricetin (DHM) is a flavonoid derived from many plants, including Hovenia dulcis. DHM has been utilized as an anti-alcohol therapeutic in Traditional Chinese Medicine for over 600 years and is sold over the counter today as a “hangover cure.”

Methods

Female and male C57BL/6J mice (n=12/group/sex) were treated with an isocaloric ethanol-containing Lieber-DeCarli diet, a widely used ibudin drinking model that closely mimics ALD pathology. Mice were randomly assigned to three groups: 1) No-EtOH; 2) EtOH (5.5% v/v); 3) EtOH (5.5% v/v) + DHM (6mg/mL). Mice receiving EtOH + DHM were administered ethanol-only for 2 weeks prior to DHM supplementation to establish the development of ALD pathology. Protein expression of PLIN1 was quantified from immunoprecipitation while PINK1, Parkin, and PLIN2 were quantified from Western Blot; all protein was isolated from liver homogenate. Immunohistochemistry was performed on liver tissue using H&E and Oil Red O staining. Adiponectin was measured from serum using a cytokine panel. Immunohistochemistry was performed on liver tissue using H&E and Oil Red O staining. Adiponectin was measured from serum using a cytokine panel. Immunohistochemistry was performed on liver tissue using H&E and Oil Red O staining. Adiponectin was measured from serum using a cytokine panel.

Results

Figure 1: DHM administration increased lipid droplet (LD) size and decreased LD count as compared to the EtOH-treatment group. Hematoxylin and Eosin (H&E) staining (A) and Oil Red O staining (B), were employed to visualize liver tissue. The H&E images showed vacuoles in white, whereas Oil Red O stained LDs bright red, as signified by black arrows. The findings revealed that DHM supplementation (C) decreased quantity of lipid droplets (*0.025) and (D) increased size as compared to the EtOH-treatment group (0.0065, ##0.0001).

Figure 2: DHM administration improved the expression of mitophagy-related proteins. Unbleached PINK1 assembles on the outer mitochondrial membrane of damaged mitochondria where it can be cleaved/activated and is one of the precursors to mitophagy. Parkin is recruited by PINK1 to mark damaged mitochondria for degradation. (A) DHM-supplementation group shows the lowest levels of uncleaved PINK1. (B) Parkin protein expression in the DHM-supplementation group is increased.

Figure 3: DHM supplementation leads to improved mitochondrial-driven respiration. (A) Oxygen consumption rate (OCR) represents mitochondrial aerobic respiration and is significantly increased in the DHM-fed group when compared to the EtOH-only group (p<0.0001, ##0.0005); (B) Extracellular acidification rate (ECAR) represents glycolysis-driven metabolism (*0.011); and (C) Adiponectin is a cytokine that has been shown to data of PLIN1 demonstrates the highest protein expression in the DHM-fed group (0.0431).

Figure 4: DHM supplementation alters perilipin composition in LD membranes. Perilipins are expressed on LD membranes and are involved in regulating their maintenance and degradation. (A) Immunoprecipitation data of perilipins demonstrates the highest protein expression in the DHM group. Increased PLIN2 expression has been shown to protect LDs from autophagy. (B) PLIN2 levels were notably lower in DHM-supplementation models.

Discussion

The prevalence of ALD and the lack of FDA-approved therapies necessitates the development of effective therapeutics. Given the hepatoprotective benefits of DHM, the present study investigated the effects of DHM supplementation on lipid accumulation and mitochondrial health in hepatic tissue. We observed an increase in lipid droplet (LD) heterogeneity and clearance with DHM administration. Additionally, DHM appeared to alter the membrane composition of LDs, indicating a shift toward larger LDs. This preliminary data suggests that DHM influences accumulation, thereby aiding in LD clearance. DHM-fed mice also show improved mitophagy health and function, efficiency of oxidative phosphorylation, and β-oxidation as shown through improved OCR, ECAR, and adiponectin levels. Additionally, results measuring mitophagy-associated proteins, PINK1 and Parkin, indicate that DHM aids in the removal of damaged mitochondria, specifically through a process called mitophagy. Mitophagy is the process of removing dysfunctional mitochondria. Taken together, this data demonstrates that DHM positively modulates mitophagy activity and LD accumulation. Mitochondrial damage and lipid accumulation are major causes of ALD progression. This evidence will support future research investigating DHM as a promising therapeutic to treat ALD by providing insight into DHM’s role in mitigating mitochondrial damage and hepatic lipid accumulation.

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