

## **Hepatocyte CD73 Mediates Alcohol-Induced Liver Injury in a Sex-Dependent Manner**

Alcoholic liver disease (ALD) caused by excessive drinking is one of the leading causes of chronic liver disease globally. Despite its significance, there is no effective treatment other than abstinence from alcohol. Combined with this clinical challenge, the fact that women have increased susceptibility to liver damage by alcohol compared to men has warranted the need to identify potential regulators in ALD that can be targeted for sex-specific diagnosis, prognosis, and treatment. One promising candidate is ecto-5'-nucleotidase (commonly known as CD73), which generates adenosine from adenosine monophosphate (AMP) in the extracellular space. We have previously shown that the loss of hepatocyte-specific CD73 (CD73-LKO) and the secondary loss of extracellular adenosine led to spontaneous sex-dependent liver injury in mice, which was exhibited as fatty liver, cell death, and inflammation. These changes are generally observed in patients diagnosed with alcoholic steatohepatitis (ASH), the most severe form of ALD with a high mortality rate. Given our observations in mice, we hypothesize that CD73 exerts sex-dependent hepatoprotective functions in alcohol-induced liver injury. In this study, primary hepatocytes were isolated from mice and treated with ethanol. CD73 mRNA and protein levels were analyzed by quantitative polymerase chain reaction and immunoblot, respectively. Male and female WT and CD73-LKO mice were fed the acute-on-chronic ethanol diet, and were compared by serological, histological, and biochemical methods. Untreated hepatocytes and standard chow-fed mice were used as controls. Our results showed that CD73 is upregulated at the mRNA and protein levels in response to ethanol in primary mouse hepatocytes. Similarly, male and female WT mice, but not CD73-LKO mice, exhibited induction of the CD73-encoding gene *Nt5e* in the liver upon alcohol consumption. In contrast to *Nt5e* expression, CD73 protein levels were altered in a sex-dependent manner, wherein male WT mice had increased levels but female WT mice had decreased levels. Mice fed the ethanol diet had increased levels of serum ALT, compared to their control-fed counterparts. In contrast to WT mice, CD73-LKO had elevated ALT levels, and was associated with increased steatosis, cell death, and inflammation, with greater severity seen in the male. In conclusion, CD73 expressed on hepatocytes is regulated transcriptionally and post-transcriptionally upon alcohol exposure. Moreover, the loss of hepatocyte-CD73 exacerbated alcohol-induced liver injury in mice, which was more prominent in male than female mice. Additional studies will reveal potential sex-dependent factors that may compensate for the loss of CD73 in female mice.