Neutrophil Extracellular Trap (NET) Formation in Experimental Acetaminophen-Induced Acute Liver Injury/Failure

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Background: Overdose on acetaminophen (APAP), one of the most common over-the-counter pain relievers, can cause acute liver injury (ALI), which may progress to fatal, acute liver failure (ALF). Therapies for ALI/ALF are limited; experimental evidence suggests neutrophils drive APAP-induced ALF. During injury or inflammation, neutrophils have been shown to release neutrophil extracellular traps (NETs), consisting of networks of extracellular DNA, citrullinated histones, and neutrophil proteins. NET markers have been detected in patients after APAP overdose, suggesting NETs may play a role in APAP-induced ALI/ALF. However, the role of NETs during APAP-induced ALI and ALF is still unknown. Purpose: The purpose of this study was to quantify the injury and neutrophil extracellular trap formation in APAP-induced ALI and ALF. Hypothesis: Intrahepatic NET formation occurs in experimental APAP-induced ALF. Methods: Formalin-fixed, paraffin-embedded (FFPE) liver tissues from mice challenged with saline or APAP (300mg/kg, 600mg/kg) for 6-12 h were sectioned and stained with hematoxylin and eosin (H&E) or immunolabeled for NETs. Quantitation of hepatocyte necrosis was performed on H&E-stained tissues using QuPath software. Immunolabeled liver sections for NET components Ly6G (neutrophil surface marker), citrullinated histone H3, and DAPI (DNA) were scanned on a fluorescence microscope and quantified using thresholding methods in QuPath software. Results: As anticipated, hepatocyte necrosis was significantly increased following APAP challenge compared to vehicle controls at 6 h and 12 h. NETs were significantly increased at both doses of APAP compared to vehicle. Conclusion: NETs were detected in experimental APAP overdose warranting further study of their contribution to liver injury. Supported by NIH R25ES020721.