The dynamics of extracellular DNA and neutrophil extracellular traps formation in mouse models of chronic liver disease

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Background: Steatosis, a common feature of many liver diseases, refers to an abnormal fat accumulation in hepatocytes. It can easily progress into steatohepatitis, characterized by inflammation, and lately fibrosis, cirrhosis, and hepatocellular carcinoma. Therefore, understanding the mechanisms driving liver damage is crucial. In inflammation, neutrophils infiltrate inflamed sites and release neutrophil extracellular traps (NETs) as a part of NETosis cell death. In this process, DNA is released into extracellular space. High concentrations of extracellular DNA (ecDNA) can exacerbate inflammation and further tissue damage, supporting the role of NETs in the progression of liver diseases. This study aimed to analyze NETs formation and ecDNA dynamics in animal models of chronic liver diseases.

Methods: Adult female C57BL/6J mice were used for inducing non-alcoholic steatohepatitis and liver fibrosis by commercial low-methionine choline-deficient diet (CDAA model) or intraperitoneal administration of hepatotoxic thioacetamide in increasing concentrations of 50-400mg/kg (TAA model). Dynamics of liver enzymes, NETs formation, ecDNA, and markers of NETosis were monitored over 10 weeks.

Results: Both models were successfully induced. A gradual increase of alanine and aspartate aminotransferases, as well as the number of neutrophils in plasma were observed. NETs formation did not differ over time, compared to control groups. Increased concentrations of ecDNA were observed in both models since the 2nd week (CDAA: $p<0.001$, TAA: $p<0.01$). Notably, ecDNA of nuclear origin (ncDNA) was increased from the 6th week in the CDAA model ($p<0.001$) and at the end of the experiment in the TAA model ($p<0.05$). No changes in ecDNA of mitochondrial origin were detected. Myeloperoxidase, a marker of NETosis, was increased from the 2nd week in the CDAA model ($p<0.01$), compared to controls.

Conclusion: The results indicate increased concentrations of ecDNA, specifically ncDNA, but no change in NETs formation over 10 weeks. However, the origin and role of ecDNA remain unknown. Increased concentrations of ecDNA could arise from not only NETotic neuropils but also damaged hepatocytes, which should be the focus of further experiments.

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