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Murine Ethanol Ingestion Promotes Hepatic Platelet Accumulation Mimicking Acute CCL_4 Exposure

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INTRODUCTION

Dysregulated coagulation contributes to inflammation and fibrosis from chemical injury. Platelets are key contributors to inflammation and are primary sources of TGF-b, PDGF, and EGF that promote fibrosis, and so may contribute to hepatic fibrosis. We questioned whether platelets accumulate during chronic ethanol- or chemical-induced hepatic injury, whether platelet accumulation would occur prior to induction of hepatic fibrotic responses, and whether platelet accumulation reflects deposition of intravascular microthrombi or individual platelets intercalated into liver parenchyma.

METHODS

We modeled acute hepatic injury with a single injection of CCl_4, a chronic model of moderate ethanol ingestion, or a combination of the two insults. C57Bl6 mice ingested a control liquid diet or provided free access to 1% ethanol (2d), then 2% ethanol (2d, 11% calories). At day 4, mice received, or not, a single i.p. injection of CCl_4 (1 µl/g, 1:3 in olive oil), with sacrifice 72h later. Formalin-fixed livers were transversely sectioned, paraffin-embedded prior to immunohistochemistry with anti platelet integrin gpIIb (CD41), endothelial CD31, or a-smooth muscle actin (aSMA) antibodies with DAPI nuclear staining. Adherent platelets spread to micron thickness, so detection was by serial tyramide amplification (Biotium). This catalyzed reporter deposition system uses a single tyramide dye activated by HRP-derived H_2O_2 to a reactive specie that multiply ligates adjacent molecules before the antibody complex is thermally stripped prior to a subsequent tyramide labeling.

CCl4 72hr no EtOH
MFT322 "15 4X CD41 (R), aSMA (G) and CD31/PEDCAM1 (Y) DAPI (B)

MFT 166#13 EtOH/olive oil
CD41 (R), aSMA (G) and CD31 (Y) DAPI (B)

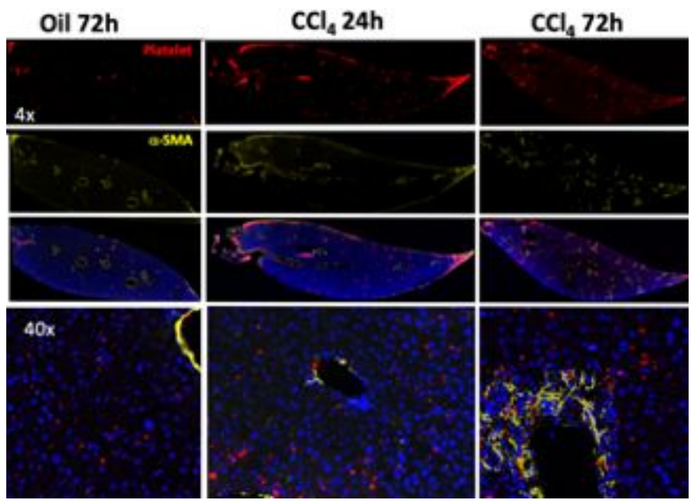

Fig. 1. Platelet and activated HSC accumulation in CCl_4 hepatotoxicity. Platelet CD41 (red) and aSMA (yellow) of activated HSC in livers of mice injected with CCl_4 or oil sacrificed 24 or 72h later. Blue, nuclear DAPI. Sequential immunohistochemistry by antigen-dependent tyramide signal amplification.

RESULTS

Our preliminary data show parenchymal platelet deposition with inflammation 24h after CCl_4 injection massively increasing platelet accumulation, with enhanced expression of aSMA, just below the outer Glisson's sheath encasement, correlating to the area of highest arterial flow (DOI 10.1139/y93-018). Platelet accumulation, but not aSMA, within liver parenchyma was modestly increased at this time. 72h after CCl_4 injection, subsurface platelet accumulation in association with endothelial cell PECAM1 remained apparent, with aSMA now extended in disordered filaments surrounding portal tracts. Ethanol ingestion alone, similar to CCl_4 exposure, revealed massive platelet accumulation just below the Glisson's sheath liver encasement in association with endothelial cell PECAM1 without a aSMA deposition. The combination of ethanol and CCl_4 presented similarly.

CONCLUSION

We conclude ethanol ingestion promotes hepatic platelet accumulation, providing a non-transcriptional source of fibrotic growth factors, that parallels hepatic injury invoked by CCl_4 exposure.