THE ROLE OF INTERLEUKIN-36 CYTOKINES IN HEPATIC ISCHAEMIA-REPERFUSION INJURY

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Introduction: Liver disease remains one of the only major causes of mortality and morbidity that is increasing across Europe. Chronic hepatic inflammation resulting from underlying liver disease leads to fibrosis, cirrhosis, and hepatic failure. The only viable treatment for patients with late-stage liver disease is liver transplant. However, one-year mortality following transplant occurs in approximately 8% of patients and around 20% require re-transplantation. This is driven, in part, by hepatic ischaemia-reperfusion injury – the paradoxical injury that occurs upon reintroduction of blood flow to a previously non-perfused organ. The Interleukin-36 (IL-36) family is a relatively novel pro-inflammatory cytokine family, with identified roles in inflammation across a range of diseases/tissues. However, the role of IL-36, the IL-36 receptor (IL-36R) and the endogenous antagonist (IL-36Ra) in the liver is poorly understood - particularly following IR injury. **Methods:** In this study, we used a murine model of hepatic IR injury to identify the role of IL-36 in this pathology. Partial hepatic ischaemia was generated in mice (C57BL/6; 8-12wk) by isolating the blood supply to the left lobe of the liver for 60 minutes using an atraumatic vascular clamp. Sham animals underwent similar surgery, but without application of the clamp. The liver was monitored using intravital microscopy, and tissues were collected for in vitro analysis using immunofluorescence, static adhesion assays, and ELISA. To examine the role of IL-36 cytokines independent of injury, we topically applied agents to the liver by affixing a small well to the liver surface into which these agents were placed. **Results:** Intravital microscopy revealed neutrophil and platelet accumulation in IR livers was significantly enhanced when compared to sham livers. Ex vivo analysis of tissue sections revealed no differences in F4/80^{+ve} or CD4^{+ve} cell counts in the IR liver when compared to sham controls. Immunofluorescence staining of IR livers revealed significantly enhanced staining for IL-36α and IL-36β when compared to sham controls; this staining appeared to be localised in both the macro- and micro-vasculature. Interestingly, we did not identify an increase in IL-36R expression in the liver following IR injury. Topical application of IL-36β on the liver led to significantly enhanced neutrophil recruitment in the treated area when compared to vehicle control. Administration of IL-36Ra into mice prior to hepatic IR led to a significant reduction in neutrophil recruitment when compared to vehicle controls. Interestingly, we observed an increase in CD4^{+ve} cell recruitment in IL-36Ra treated IR mice compared to controls, likely due to preserved vascular perfusion. **Conclusion:** Levels of IL-36 cytokines (but not the receptor) are significantly enhanced during hepatic IR. Administration of an IL-36 receptor antagonist (IL-36Ra) significantly reduces neutrophil recruitment during IR injury. Agents targeting IL-36 family members may have potential therapeutic efficacy by limiting inflammation and thus the downstream tissue damage that occurs during IR injury.