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Acetaminophen-induced liver injury in mice can be alleviated by reducing hepatic TGFβ1 signaling

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Acetaminophen (APAP)-induced hepatotoxicity is the leading cause of acute liver failure in the United States and Western Europe with mortality approaching 30%. We have previously identified that transforming growth factor beta 1 (TGFβ1) is upregulated in a mouse model of hepatic encephalopathy due to acute liver failure and TGFβ1 contributes to the pathology of this syndrome. In order for TGFβ1 to induce its effects, it must signal through a heterotetramer receptor complex made up of TGFβ receptor 1 and TGFβ receptor 2, which leads to the phosphorylation of SMAD2 and SMAD3. At this time, little is known about the role of TGFβ1 during APAP-induced liver failure. We hypothesize that TGFβ1 exacerbates hepatic damage and pathology associated with APAP-induced hepatotoxicity.

Methods: Male C57Bl/6 mice or hepatocyte specific TGFβ1 null mice ($TGF\beta1^{-/-}$) were fasted for 12 hours prior to APAP injection and at time points up to 48 hours after injection, livers and serum were collected. In parallel, APAP-treated mice and saline-injected controls were pretreated with GW788388, a TGFβ receptor 1 antagonist, for 1 hour prior to APAP injection. Liver histology was assessed by hematoxylin & eosin staining and liver function was determined by ALT and AST measurement. Hepatocyte necrosis was calculated by measuring the area devoid of nuclei in H&E stained liver sections. TGFβ1 and SMAD2/3 expression were assessed by immunoblotting, immunohistochemistry and/or qPCR. Glutathione was measured using a commercially available kit and oxidative stress was determined by performing immunoblots for SOD1 and iNOS.

Results: Mice injected with APAP had elevations of hepatic and circulating TGFβ1 as well as an increase in the phosphorylation of SMAD3 with no significant change in SMAD2 expression. $TGF\beta1^{-/-}$ mice treated with APAP were found to have reduced SMAD3 phosphorylation compared to C57Bl/6 controls. APAP-treated mice pretreated with GW788388 had elevated TGFβ1 but had reduced phosphorylation of SMAD3. $TGF\beta1^{-/-}$ or GW788388 pretreated mice had reduced APAP-induced hepatic damage and improved liver function as determined by reduced ALT and AST levels. Increased glutathione levels were present in liver homogenates from APAP-treated mice with levels being reduced in $TGF\beta1^{-/-}$ mice or by pretreatment with GW788388. Oxidative stress that was elevated in APAP-treated mice was reduced in APAP-treated $TGF\beta1^{-/-}$ and mice GW788388 pretreated mice.

Conclusion: Elevated TGFβ1 following APAP-induced liver failure contributes to increased phosphorylation of SMAD3, which increases liver damage and oxidative stress. These deleterious effects were reversed by eliminating hepatocyte TGFβ1 signaling via genetic ablation or GW788388 pretreatment, indicating that TGFβ1 signaling could be a potential therapeutic target for APAP-induced hepatotoxic liver injury.

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