**ABSTRACT**

**Background:** Cholestatic liver diseases, including Primary Sclerosing Cholangitis (PSC), are characterized by severe portal inflammation with progression to fibrosis and cirrhosis and ultimately liver failure. Recently, we have determined that the thioredoxin antioxidant response is dysregulated during PSC contributing to an increase in hepatocellular oxidative injury. The objective of this study was to examine the impact of inhibition of thioredoxin reductase 1 (TrxR1) on hepatic inflammation and liver injury during acute cholestasis.

**Methods:** Using primary hepatic mouse macrophages, as well as liver tissue from WT/TrxR1LKO mice that have undergone sham or bile duct ligation +3 days, we analyzed the effect of inhibition of TrxR1 on expression of the Nlrp3 inflammasome, proinflammatory cytokines as well as inflammation and liver injury. **Results:** Exposure of mouse primary intralobular hepatocytes, bone marrow-derived, and RAW264.7 macrophages to TNF/ILPS resulted in increased expression of TrxR1, Nip3, Il1b and Il18. Co-incubation with the TrxR1 inhibitors auranofin and Tri-1 ameliorated TNF/ILPS induced Nip3 inflammasome activation and mRNA expression of proinflammatory cytokines. Immunohistochemical analysis of TrxR1 revealed increased expression in resident macrophages in hepatic tissue isolated from bile duct ligated mice as well as human cholestasis supporting in vitro data. Compared to the Sham controls, WT BDL mice exhibited increased inflammation, necrosis and expression of proinflammatory cytokines (Il1b, Il18, TNFα) and the Nip3 inflammasome complex (Nip3, ASC, GSDMD) which was ameliorated in TrxR1LKO mice. **Conclusion:** These data support thioredoxin reductase signaling as a critical regulator of Nlrp3 inflammasome activation and proinflammatory cytokine production in macrophages during cholestasis and suggest that targeting thioredoxin reductase activity may have potential therapeutic benefit during cholestatic liver disease.

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**RESULTS**

**Background:** Chronic cholangiopathies including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and biliary atresia are diseases of unknown etiology characterized by extensive bile duct destruction with the bile ducts.

• Account for ~16% of all liver transplants and 50% of pediatric transplants (biliary atresia) that were performed in the US from 1988-2014.

• With only poor therapeutic options available, there is an urgent need for an improved mechanistic understanding of these diseases so that new strategies can be developed.

• In the liver, inflammasomes are important innate immune sensors that assist in maintaining cellular function in response to pathogenic or stress signals.

• The NLR Family Pyrin Domain Containing 3 (Nlrp3) inflammasome complex has been shown to be upregulated in human and murine cholestatic liver disease and plays an integral role in regulating hepatic inflammation and fibrosis by increasing production of proinflammatory cytokines (IL1β, IL18).

• Recently, members of the thioredoxin redox pathway (TrxR1, TrxR2) and TrxR1LKO mice have emerged as important mediators of Nlrp3 activation.

**Abstract:** Thioredoxin Reductase 1 is a critical mediator of hepatic inflammation during cholestatic liver injury

**Background:**

In human and murine cholestasis, thioredoxin reductase is upregulated in macrophages and in portal hepatocytes surrounding areas of increased inflammation.

• The thioredoxin reductase inhibitor auranofin ameliorates LPS/TNF induced expression of Nip3 and proinflammatory cytokines and upregulates thioredoxin in hepatic macrophages.

• In mice, acute cholestatic injury (BDL) results in inflammasome activation.

• TrxR1LKO decreases neutrophil infiltration, necrosis and increased expression of Nipr3 inflammasomes/proinflammatory cytokines following BDL+3d.

• Liver specific (LKO) deletion of TrxR1 decreases Nrf2 response genes but does not impact oxidative stress following BDL+3d.

**Figure 1.** Inhibition of TrxR1 prevents LPS/TNF induced expression of Nip3, Il1b and Il18. Intrahepatic mononuclear cells were incubated with LPS/TNF (100ng/ml/10ng/ml 4hr) +/- the TrxR1 inhibitor auranofin (1µM, 3µM, 5µM). mRNA was isolated and qPCR analysis of Nip3, Il1b, Il18, TrxR1 and TrxR2 examined. mRNA was normalized to Hprt expression. Values are Mean± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

**Figure 2.** Liver specific (LKO) deletion of TrxR1 significantly reduces liver injury following bile duct ligation+3d. A. AST B. ALT. Values are Mean± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

**Figure 3.** Liver specific (LKO) deletion of TrxR1 significantly reduces neutrophil but not T-lymphocyte or macrophage infiltration following BDL+3d. A. F4-80+ macrophages B. Myeloperoxidase (MPO)+ neutrophils C. CD3+ lymphocytes. PT=Portal triad, CV-Central vein

**Figure 4.** Liver specific deletion of TrxR1 significantly decreases bile duct injury, hepatic necrosis, but has no effect on fibrosis or portal proliferation A. Hematoxylin and eosin (H&E) B. Picrosirius red (PSR) C. Cytokeratin 7 (CK7) PT=Portal triad, CV-Central vein, areas of necrosis are in red. ***p<0.0001.

**Figure 5.** Liver specific deletion of TrxR1 significantly reduces mRNA expression of Nip3, Il1b, Il18, TrxR1 and Nlrp3 during acute cholestasis. Values are Mean± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

**Figure 6.** Liver specific (LKO) deletion of TrxR1 significantly reduces Nlrp3 inflammasome mRNA expression. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

**Figure 7.** Liver specific (LKO) deletion of TrxR1 significantly reduces mRNA expression of Nip3 and proinflammatory cytokines following BDL+3d. mRNA was normalized to Hprt expression. Values are Mean± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

**Figure 8.** Liver specific (LKO) deletion of TrxR1 induces Nrf2 response genes but does not impact oxidative stress following BDL+3d. Immunohistochemical analysis of 4-hydroxyethylpyruvate (4-HNE), p38, Carbonyl reductase 3 (CBR3), GSTmu, Heme Oxygenase 1 (HO1)-PT Portal triad, CV-Central vein

**CONCLUSIONS AND FUTURE DIRECTIONS**

- In human and murine cholestasis, thioredoxin reductase is upregulated in macrophages and in portal hepatocytes surrounding areas of increased inflammation.

- The thioredoxin reductase inhibitor auranofin ameliorates LPS/TNF induced expression of Nip3 and proinflammatory cytokines and upregulates thioredoxin in hepatic macrophages.

- In mice, acute cholestatic injury (BDL) results in inflammasome activation.

- TrxR1LKO prevents neutrophil infiltration, necrosis and increased expression of Nipr3 inflammasomes/proinflammatory cytokines following BDL+3d.

- Liver specific (LKO) deletion of TrxR1 induces Nrf2 response genes but does not impact oxidative stress following BDL+3d.

The Nipr3 inflammasome complex plays a critical role in the pathogenesis of cholestatic liver disease. Targeting thioredoxin reductase pathway mediated inflammasome activation may provide therapeutic benefit in combination with other targeted therapies. Future studies will be focused on understanding the cell specific contribution of the thioredoxin pathway inflammasome activation and hepatic injury during cholestasis.