Hepatic circadian rhythms are dysregulated by increased cytokine production in mice subjected to concomitant intestinal injury and parenteral nutrition

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ABSTRACT

Background: We have developed a mouse model of Parenteral Nutrition Associated Cholestasis (PNAC) in which combining intestinal inflammation and PN infusion results in cholestasis, hepatic macrophage activation, and transcriptional suppression of bile acid signaling. In the liver, the master genes Clock and Arntl/Bmal drive rhythmic gene expression and regulate circadian expression of hepatic functions including bile acid synthesis. Once activated, Bmal/Clock are negatively regulated by several transcription factors including Nr1d1, Dbp, Dec1/2, Cry1/2 and Per1/2. The aim of this study was to examine the expression of hepatic functions including bile acid synthesis. Once activated, Bmal/Clock are negatively regulated by several transcription factors including Nr1d1, Dbp, Dec1/2, Cry1/2 and Per1/2. The aim of this study was to examine the expression of hepatic functions including bile acid synthesis.

Methods: First, the effects of i.p. administration of recombinant IL-1β or TNFα on CR on the liver was examined. Second, WT, Il1b−/− or Tnfr1/2−/− mice were exposed to dextran sulfate sodium (DSS) for 4 days followed by soy-oil lipid emulsion-based PN infusion through a central venous catheter for 14 days (DSS-PN). Associated Cholestasis (PNAC) in which combining intestinal inflammation and parenteral nutrition was defined by increased serum aspartate aminotransferase, alanine aminotransferase, total bile acids, and total bilirubin.

Results: Intraperitoneal injection of IL-1β or TNFα into WT mice suppressed mRNA expression of Nr1d1, Amtl and Clock and increased Dbp and Per2. In the PNAC model, WT DSS-PN increased serum biomarkers of hepatic injury (ALT, AST, serum bile acids) which was suppressed in both DSS-PN IL1bKO and DSS-PN Tnfr1/2KO groups. Interestingly, administration of PN in the absence of DSS increased Amtl but had no effect on Per2 or Dbp.

Conclusions: Disruption of CR in part due to gut-derived cytokine (IL-1β and TNFα) production in murine PNAC. Administration of PN in the absence of gut injury has opposing effects on circadian rhythms. Pharmacologic targeting of CR as a therapeutic strategy for PNAC thus deserves further investigation.

Figure 2. Dysregulation of Circadian Rhythms in murine PNAC. mRNA expression of Bmal1 and Clock was suppressed in both DSS-PN IL1β−/− and DSS-PN Tnfr1/2−/− mice. Expression was normalized against HPRT. Values are Mean±SEM. *p<0.05. **p<0.01.

Figure 3. Increased IL-1β and TNFα suppress hepatic circadian rhythms. 8-10 wk old C57BL/6 mice were injected with recombinant IL-1β (200ng/mouse) or TNFα (200ng/mouse). Liver tissue was harvested, and transcription factors regulating hepatic CR analyzed by qPCR. Expression was normalized against HPRT. Values are Mean±SEM. *p<0.05. **p<0.01.

Figure 4. Deletion of IL-1β signaling restores hepatic circadian rhythms following DSS-PN. 8-10 wk old IL1β−/− were subjected to Chow or 14d DSS-PN. Liver tissue was harvested, and transcription factors regulating hepatic CR analyzed by qPCR. Expression was normalized against HPRT. Values are Mean±SEM. *p<0.05. **p<0.01, ***P<0.001.

Figure 5. Deletion of IL1 or TNFα signaling restores ameliorates liver injury DSS-PN. Deletion of Tnfr1/2 mitigates hepatic cholestasis induced by steatorrhea sodium parenteral nutrition (DSS-PN). Wild-type (WT) or TNFR1/2KO mice were treated with chow or DSS-PN and euthanized after 14 days. Serum measures of hepatic injury and cholestasis were obtained and compared (Alamine aminotransferase (ALT), Aspartate aminotransferase (AST), Total serum bile acids). (A) IL1β−/− B. TNFR1/2−/−. Values are Mean±SEM. ***p<0.001. ****P<0.0001.

CONCLUSIONS

• mRNA Expression of Bmal is suppressed by DSS-PN corresponding to an increase in Per2, Ddb and Dec2.
• Proinflammatory cytokines IL-1β and TNFα both suppress mRNA expression of Bmal, Npas2, Clock, and increase Per2, Cry2, Ddb and Dec2.
• Deletion of IL1 prevents DSS-PN-mediated suppression of circadian rhythms.
• Deletion of TNFR1/2 prevents DSS-PN-mediated suppression of circadian rhythms.