Perinatal Acetaminophen Toxicity Is Mediated by Cytochrome P450 2e1 (Cyp2e1) in a Time and Dose Dependent Manner

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Rationale
Acetaminophen (APAP) exposures occur in 50-60% of pregnancies in the US and is associated with childhood respiratory morbidity. The mechanism behind this remains unknown. Toxicity is dependent on cell-specific expression of *Cyp2e1,* the enzyme responsible for metabolizing APAP into the mitochondrial toxin NAPQI. In the developing murine lung, prenatal pulmonary *Cyp2e1* expression peaks during the saccular stage (E17.5-P4). We hypothesize this peak in *Cyp2e1* expression predicts susceptibility to APAP-induced lung injury during this developmental period and that its expression is dose dependent.

Methods
To confirm dynamic *Cyp2e1* expression in the developing murine lung, RNA was isolated from lungs of C57/B6 mice from E12-P7 and assessed for *Cyp2e1* expression. CYP2E1 protein was characterized by western blot.
C57BL/6 murine dams (n= 8-16 per condition) were exposed to APAP (either 150 or 250 mg/kg IP; 6h) on embryonic day 17 (E17) or 18 (E18). We interrogated a pulmonary transcriptional response in inflammatory (*Il6* and *mmp9*), oxidative stress (*Gclc*, *Hmox1*, *Nqo1*) and apoptotic related factors (*Trp53*, *Puma*, *Noxa*) by qPCR.

Results
In the time interval we analyzed, *Cyp2e1* expression abruptly peaks on E18 in both males and females. *Cyp2e1* protein was detected by western blot at both e17 and e18. Following APAP exposure of pregnant dams at E17 and E18, pulmonary *Cyp2e1* expression increases in males at E17 and E18 and females at E17 only ($p<0.05$), however *Cyp2e1* expression is significantly greater with exposure at E18 compared to E17. In E18, 250 mg/kg APAP exposed embryos we observed increased expression of NRF2 target genes indicative of oxidative stress: *Gclc*, *Hmox1* and *Nqo1* and induction in p53 mediated pro-apoptotic genes: *Puma* and *Noxa,* and in the inflammatory response gene *Mmp9*. Expression did not increase in similarly exposed E17 embryos. Noting the response at E18 to 250 mg/kg APAP, we explored responses at this gestational age at lower a dose of APAP. The fetal pulmonary transcriptional response to maternal APAP exposure of 150 mg/kg at E18 was attenuated compared to the 250 mg/kg dose.

Conclusions

Using a murine model, we demonstrated that pulmonary *Cyp2e1* expression is developmentally regulated, peaking on E18. We also found a dose-dependent upregulation of expression of genes associated with antioxidant response elements, apoptosis, and inflammation with maternal APAP exposure at E18. Continued work is needed to determine whether perinatal APAP exposure has detrimental effects on the developing lung, its function, and the role of pulmonary *Cyp2e*1 in this mechanism of lung injury.