Comparison of 4-ipomeanol pneumotoxicity in wild type and Cyp4b1 knockout mice

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Abstract

The cytochrome P450 super-family(CYP) is a group of enzymes that are involved in drug metabolism, bio-activation, and oxidation of organic compounds. The gene *CYP4B1* belongs to the CYP4 sub-family of P450s and codes for Cytochrome P450 4B1 protein. 4-Ipomeanol (IPO) is a furanoterpene pneumotoxin that is naturally produced by sweet potatoes (*Ipomoea batatas*) in response to infection by the fungus *Fusarium solani*. Previous studies suggest that CYP4B1 is responsible for bio-activation of IPO to a reactive toxic species in rat. In this study, we are investigating the toxicity of 4-Ipomeanol in mice with targeted disruption of the *Cyp4b1* gene.

Introduction

- 4-Ipomeanol is a pulmonary toxin in animals and a hepatic toxin in humans which is produced by sweet potatoes (*Ipomoea batatas*) infected with *Fusarium solani* fungus. Cattle and other mammals like rabbits experience extreme respiratory distress when exposed to ipomeanol, including but not limited to pulmonary edema and congestion, often leading to death(1). 4-Ipomeanol is a pro-toxin that requires metabolic activation to elicit damage (1,4,2).
- The cytochrome P450 super-family (CYP) is a group of enzymes that are involved in drug metabolism, bio-activation, and oxidation of exogenous and endogenous compounds. The gene *Cyp4b1* encodes for Cytochrome P450 4B1 protein. *Cyp4b1* is expressed in the lungs of both male and female mice, and the kidneys of male mice. The expression of CYP4B1 in the kidneys is regulated by androgens

Methods

- We purified DNA from mice tails and performed PCR. Lac Z primer – with sequences LacZ- 111 FOR Primer: 5'TAA TAG CGA AGA GGC CCGC3' and lacZ-611 REV Primer: 5'CGC CAC ATA TCC TGA TCT TCC3'– and Cyp4b1– with sequences FOR Primer: 5' GGC AAG GAG CAA AAA TGA TA3' and REV Primer: 5'CAC AGA AAT GTG TTG CCA AG3'– were used for genotyping.
- The expression of Cyp4b1 protein in lungs, livers, and kidneys of both male -/- (Knockout) and +/+ (Wild type) and female -/- and +/+ mice was confirmed using western blot. Goat α 4B1 primary and donkey α goat secondary antibody were used to visualize the 4B1 protein using actin as a loading control.
- We performed a paraffin fixation on Cyp4b1 +/+ and -/- mice lungs, liver and kidney from animals treated with 20 kg/mg of 4-Ipomeanol.
- Our toxicology study designed in the following manner:
  - 4 mice per group (Male -/-, Male +/+, Female -/-, Female +/+)
  - Study 1: 5 mg/kg Ipomeanol. Sacrifice at ~24 hours.
  - Study 2: 20 mg/kg Ipomeanol (LD50). Sacrifice at ~13 hours.

Results

- Study 1: No overt signs of toxicity. Mice appeared healthy (data not shown).
- Study 2: +/+ mice were in distress including short quick breathing within a few hours after dosing. By 13 hours they were essentially non-responsive compared to -/- mice.

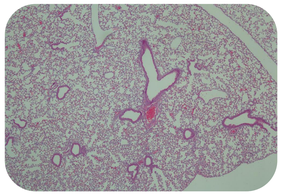

Figure 1: Histology of KO mouse lung treated with 20kg/mg Ipomeanol (4x magnification)

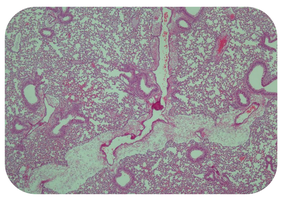

Figure 2: Histology of WT mouse lung treated with 20kg/mg Ipomeanol, visible signs of toxicity (4x magnification)

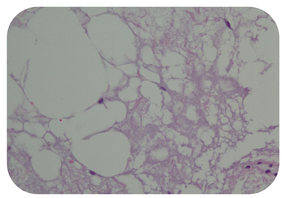

Figure 3: Histology of WT mouse lung, Ipomeanol treated, fibrins are seen to break down (40x magnification, same sample as Figure 2&4)

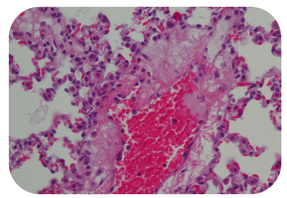

Figure 4: Histology of WT mouse lung, Ipomeanol treated, edema fluid is see to build up (40x magnification, same sample as Figure 2&3)

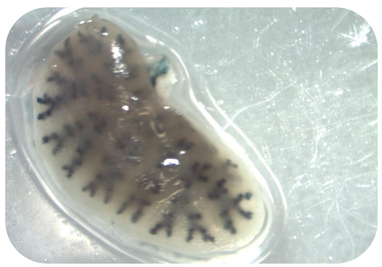

Figure 5: Whole Mount- Mouse lung lobe from Cyp4b1 knockout mice stained with the chromogenic substrate x-gel.

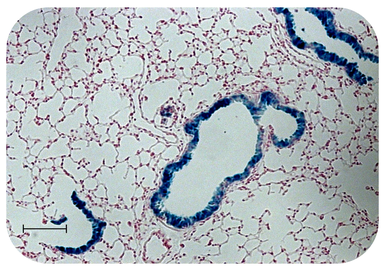

Figure 6: Magnification of figure 5. Note that staining/Cyp4b1 expression is restricted to bronchioles and absent from alveoli. (10x magnification)

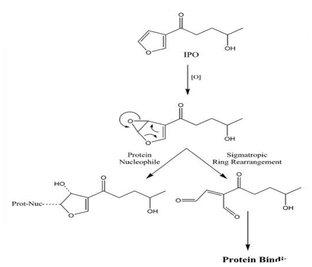

Figure 7: Ipomeanol Metabolic Scheme. Cyp4b1 Oxidizes 4-Ipomeanol into a nucleophile and following a ring rearrangement it seizes to be latent chemical and becomes toxic and causes damage by binding to proteins.

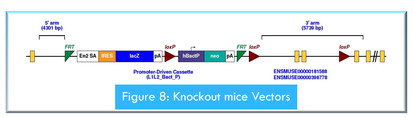

Figure 8: Knockout mice Vectors

The target vector was constructed by CSD as part of the NIH knockout mouse project (KOMP). Embryonic stem cells with targeted integration of the vector were used to generate chimeric and subsequently knock out mice. The vector contains a Lac Z reporter, which is expressed ender the control of the Cyp4b1 gene promoter.

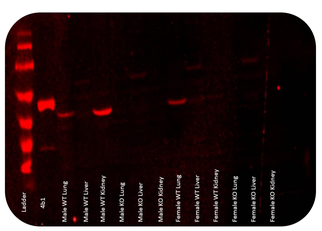

Figure 9: Western Blot

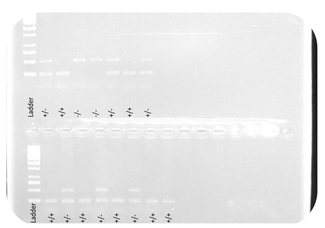

Figure 10: Genotype Gel

Discussion

- The data lead us to conclude that CYP4B1 is essential for the bio-activation of 4-ipmeanol. Without activation by CYP4B1, 4-Ipomeanol is a non-toxic. CYP4B1 catalyzes the addition of an oxygen molecule to the otherwise stable furan, this causes 4-Ipomeanol to undergo sigmatropic rearrangement to a reactive ene-dial intermediate which can bind to cellular nucleophiles and lead to damagae. In *Cyp4b1* knockout mice the absence of CYP4B1 leaves 4-Ipomeanol as a latent toxin, hence no lung toxicity. This explains why ipomeanol-treated knockout animals show no signs of toxicity while treated wild-type animals experience pulmonary edema.

Acknowledgements

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Future Direction

- Explore the role of Cyp4b1 in metabolism of other proposed substrates, including signaling molecules involved in ocular wound healing and inflammation and the purported bladder carcinogen 4-aminobipheyl.

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