

CHARACTERISATION OF THE HEPATIC EFFECTS OF PREGNENOLONE 16 α -CARBONITRILE (PCN) IN PREGNANE X RECEPTOR (PXR, NR1I2) KNOCKOUT RATS

Mark Chamberlain¹, Audrey Vardy¹, John Foster² & Clifford Elcombe¹

¹CXR Biosciences Ltd, Dundee, United Kingdom
and ²ToxPath Sciences Ltd, Congleton, UK

Introduction

The administration of some xenosensing nuclear receptor activators to rats and mice induces hepatomegaly that is characterised by hepatocellular hypertrophy (due to the proliferation of smooth endoplasmic reticulum and concomitant induction of enzymes belonging to the CYP2B and 3A families) and hyperplasia (increased semi-conservative DNA synthesis and cell proliferation).

Previous studies utilising Constitutive Androstane Receptor (CAR, NR1I3) knockout (KO) rats and mice or Pregnane X Receptor (PXR, NR1I2) KO mice have demonstrated that the presence of an active receptor is necessary for the induction of hepatomegaly.

This study was designed to investigate the effects of the PXR-activator pregnenolone-16 α -carbonitrile (PCN) in PXR KO rats.

Experimental

Male Wild Type (WT) and PXR KO rats (SAGE Labs, Boyertown, PA. n = 5 per group), were subcutaneously implanted with osmotic pumps containing BrdU to allow determination of replicative DNA synthesis (S-phase), and were administered PCN (100 mg/Kg body weight) by daily oral gavage for 7 days. Control rats of each strain received corn oil vehicle alone.

After 7 days, the rats were euthanased and livers removed and weighed. Terminal blood was taken for the determination of plasma PCN concentrations and clinical chemistry parameters. Pieces of liver were taken for fixation and blocking (sections for H&E and BrdU immunohistochemistry), mRNA isolation (Taqman qPCR and microarrays) and the isolation of microsomes.

Microsomal monooxygenase activities determined were:

ethoxyresorufin-*O*-deethylation (EROD, CYP1A), pentoxyresorufin-*O*-depentylation (PROD, CYP2B), benzyloxyresorufin-*O*-depentylation (BROD, CYP2B) and benzyloxyquinoline-*O*-debenzylation (BQ, CYP3A).

One colour microarray analysis of mRNA isolated from control and treated rat liver samples was performed to facilitate generation of differentially expressed gene signature lists (DEGs) to identify gene changes in response to PCN exposure in either WT or PXR KO rats. Ingenuity pathway analysis (IPATM) software was used to identify canonical pathways.

Results

PCN administration had no effect on body weight or bodyweight gain in WT or PXR KO rats.

Terminal plasma concentrations of PCN were approximately 0 and 0.3 μ g/ml in WT and PXR KO rats respectively.

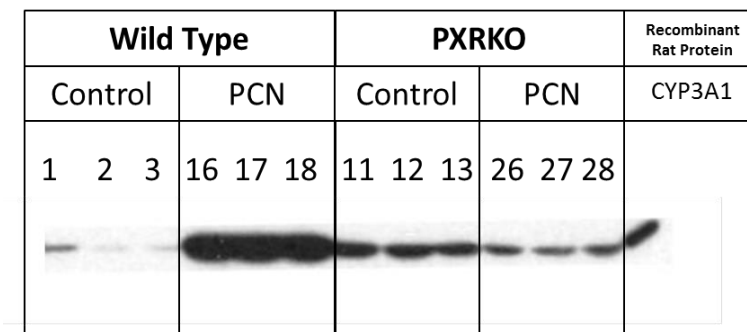
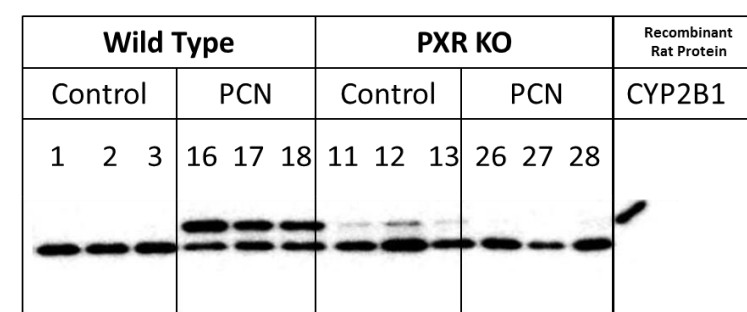
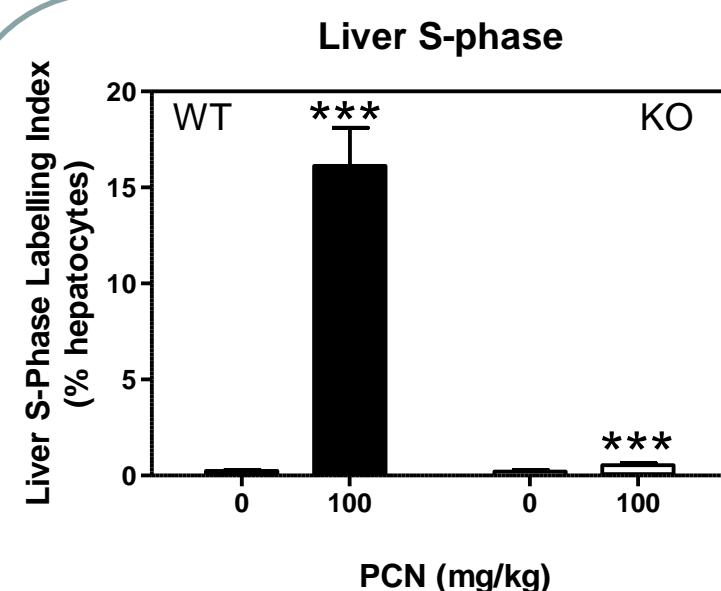
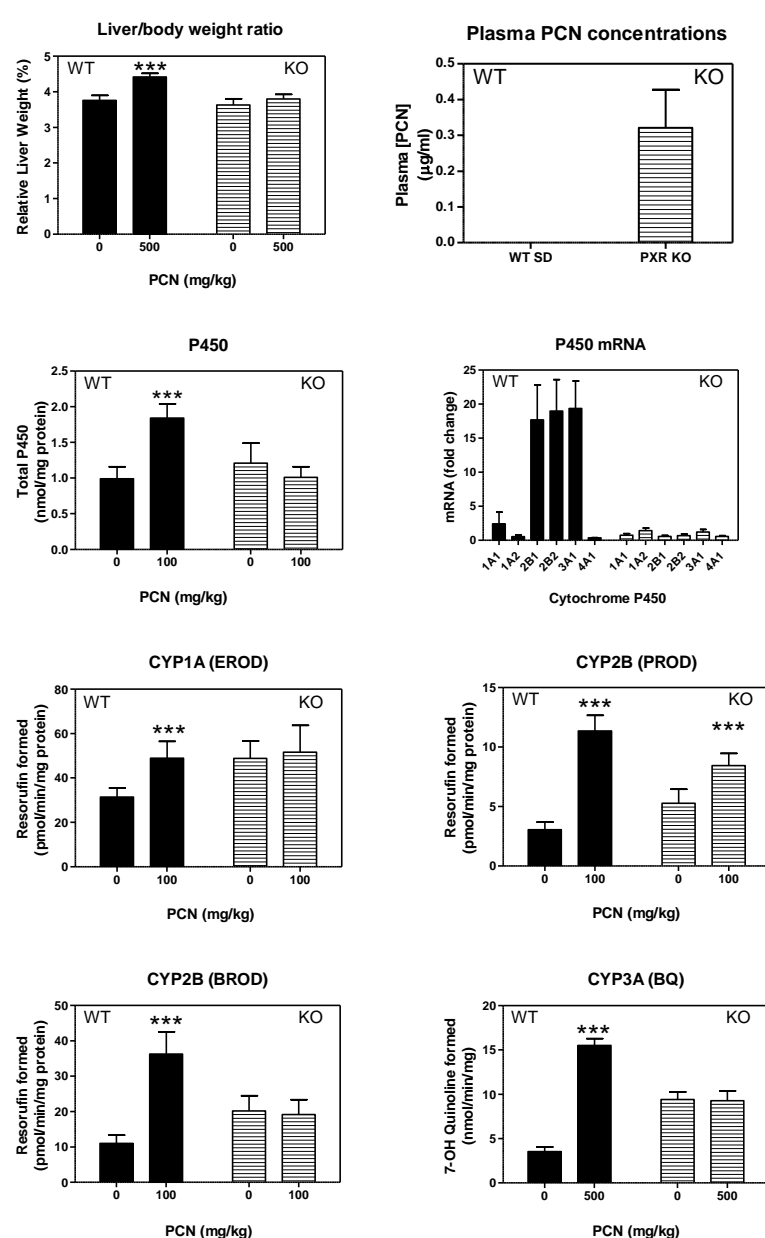
There were no PCN - related changes in ALT, AST, or ALP.

PCN-treatment of WT rats, but not PXR KO rats, resulted in 1.2 - fold increases in liver weight and liver/body weight ratios. Centrilobular hepatocellular hypertrophy was not observed in the livers of PCN-treated WT rats or PXR KO rats.

A 5-fold increase in S-phase DNA synthesis was observed in the livers of PCN-treated WT rats but not in PXR KO rats.

PCN-treatment of WT rats, but not PXR KO rats, resulted in a 1.9 - fold increase in total microsomal cytochrome P450 and 4 - fold and 3 - fold induction of PROD and BROD respectively. BQ was induced approximately 4 - fold in WT rats.

The differential PCN-mediated induction of enzyme activities between WT rats and PXP KO rats was reflected by marked differences in the expression of P450 mRNAs and proteins.

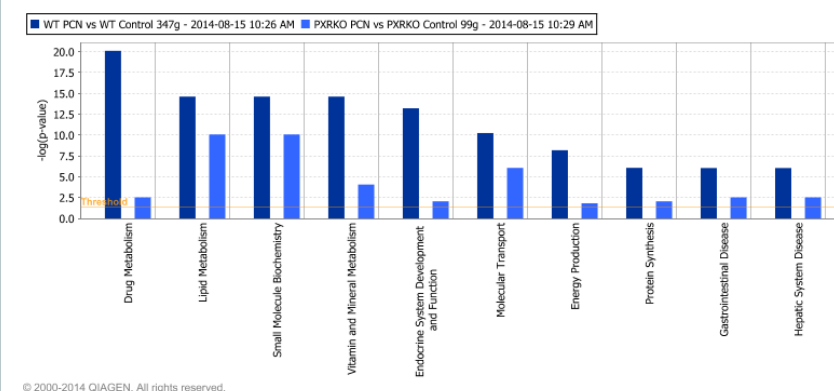


Bioinformatics and Pathway Analysis

- 347 differentially expressed genes were noted in the PCN-treated WT rats *versus* control WT rats (selection criteria: >1.5 fold, p < 0.05).
- 99 differentially expressed genes were noted in the PCN-treated PXR KO rats *versus* control PXR KO.

Top 10 Biological Functions: WT PCN-treated *versus* WT control rats and PCN-treated PXR KO *versus* PXR KO control rats.

Analysis: WT PCN vs WT Control 347g - 2014-08-15 10:26 AM



© 2000-2014 CIAGEN. All rights reserved.

Conclusions

- Induction of CYP3A1 and CYP2B in rats by PCN is due to the activation of PXR and not CAR.**
- 72% of the genes regulated by PCN appeared to be PXR-dependent.**
- An active PXR is required for PCN-mediated hepatomegaly, enzyme induction and cell proliferation in rats.**