

Mark P Chamberlain, Corinne Haines and Clifford R Elcombe
CXR Biosciences Ltd, Dundee, UK,

Introduction

The administration of Constitutive Androstane Receptor (CAR, NR1I3) 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 CAR knockout (KO) mice have demonstrated that the presence of an active CAR is necessary for the induction of this hepatomegaly. This study was designed to investigate the effects of the CAR-activator phenobarbital (PB) in CAR KO rats.

Experimental

Male Wild Type (WT) and CAR 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 PB (500 ppm) in the diet for 7 days. Control rats of each strain received diet alone.

After 7 days, the rats were euthanased and livers removed and weighed. Terminal blood was taken for the determination of plasma PB 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), 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 phenobarbital exposure in either WT or CAR KO rats. Ingenuity pathway analysis (IPA™) software was used to identify canonical pathways.

Results

PB administration had no effect on body weight or bodyweight gain in WT or CAR KO rats.

The average daily achieved dose of PB was 31.3 mg/kg and 30.8 mg/kg in WT and CAR KO rats respectively. Terminal plasma concentrations of PB were $8.86 \pm 4.70 \mu\text{g/ml}$ and $43.32 \pm 8.73 \mu\text{g/ml}$ in WT and CAR KO rats respectively.

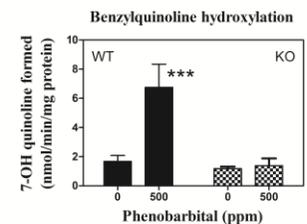
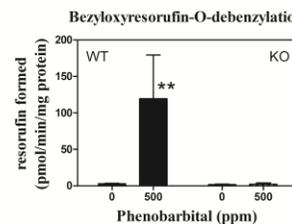
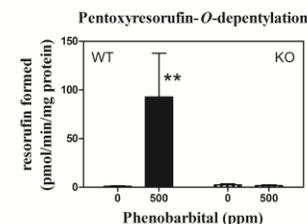
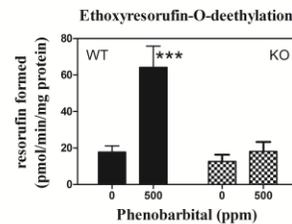
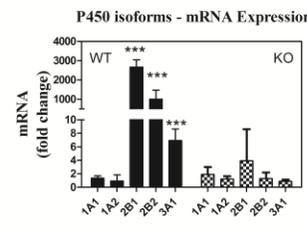
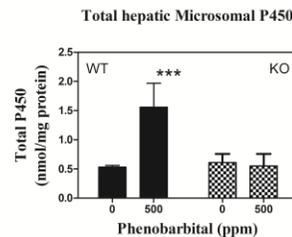
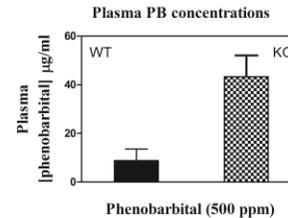
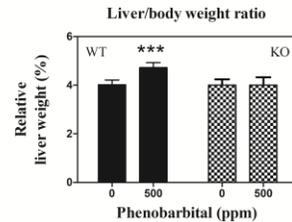
There were no PB - related changes in ALT, AST, ALP, glucose, triglycerides, HDL or LDL.

PB-treatment of WT rats, but not CAR KO rats, resulted in 1.2 - fold increases in liver weight and liver/body weight ratios that were reflected in the observation of moderate to marked centrilobular hepatocellular hypertrophy in the livers of PB-treated WT rats, but not in the livers of treated-CAR KO rats.

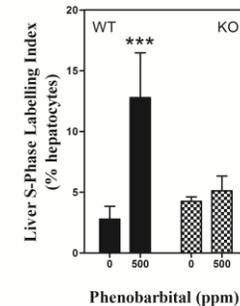
A 5-fold increase in S-phase DNA synthesis was observed in the livers of PB-treated WT rats.

PB-treatment of WT rats, but not CAR KO rats, resulted in a 3 - fold increase in total microsomal cytochrome P450 and 100 - fold and 40 - fold induction of PROD and BROD respectively. BQ was induced approximately 3 - fold.

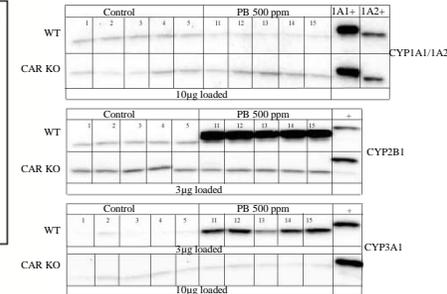
The differential PB-mediated induction of enzyme activities between WT rats and CAR KO rats was reflected by marked differences in the expression of P450 mRNAs and proteins.



Liver S-Phase

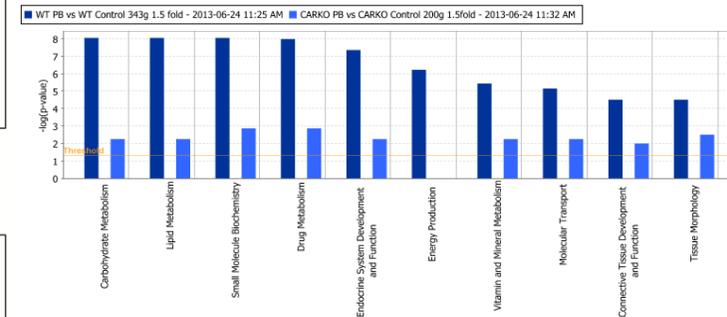


Western Blotting



Bioinformatics and Pathway Analysis

- 343 differentially expressed genes were noted in the PB-treated WT rats *versus* control WT rats (selection criteria: >1.5 fold, p < 0.05).
- 200 differentially expressed genes were noted in the PB-treated CAR KO rats *versus* control CAR KO rats.



Top 10 Biological Functions: WT PB-treated *versus* WT control rats and PB-treated CAR KO *versus* CAR KO control rats.

Conclusions

- Induction of CYP3A1 in rats by PB is due to the activation of CAR and not PXR.
- Only 40% of the genes regulated by PB appeared to be CAR-dependent.
- An active CAR is required for phenobarbital-mediated hepatomegaly (hepatocellular hypertrophy and hyperplasia) in rats.

Values are Mean \pm SD (n=5). A Student's t-test (2-sided) was performed on the results; *statistically different from control p<0.05; **p<0.01; *** p<0.001.