

# Human Constitutive Androstane Receptor (CAR) Supports The Hypertrophic But Not The Hyperplastic Response To The Murine Non-Genotoxic Carcinogen Phenobarbital (PB) *In Vivo*.

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## Introduction

Phenobarbital (PB) is a non-genotoxic carcinogen that in mice induces hepatomegaly (characterised by hypertrophy and hyperplasia) and, following long-term treatment, hepatocellular tumours, possibly due to its ability to increase cell proliferation. The relevance of these tumours to human health is controversial due to the lack of a clear molecular mechanism and suitable human-like models.

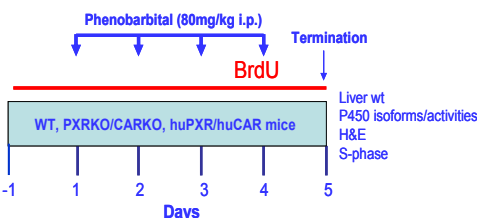
PB has been shown to activate the murine and human constitutive androstane receptors (CAR) and pregnane X receptors (PXR)<sup>(1-2)</sup>. The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. CAR knockout mice cannot activate the Cyp2b10 gene in response to PB, nor do the hypertrophic and hyperplastic responses elicited by PB occur<sup>(3)</sup>.

**Are hyperplastic responses to chemicals observed in animals relevant to humans and what are the molecular mechanisms behind these species differences?**

## Methods

PB (80 mg/kg ip., 4 days) was administered to double "humanised" CAR and PXR (huPXR/huCAR) mice and wild type C57BL/6J mice to investigate whether hyperplastic responses to chemicals observed in rodents are relevant to humans. Mice which were devoid of both receptors (PXRKO/CAR KO) were used as controls. Mice (n = 10 per group) were implanted with osmotic pumps containing BrdU to allow determination of replicative DNA synthesis (Fig. 1).

## Fig 1: Experimental Design

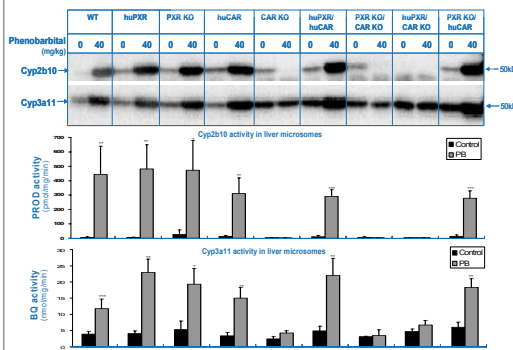


## Results

Relative liver weights were increased (to approximately 120% of controls) by PB in the wild type (WT) mice and "humanised" mice but not in the PXRKO/CAR KO animals. Cytochrome P450 induction was observed in WT and huPXR/huCAR mice but not in the PXRKO/CAR KO mice, as determined by catalytic activity measurements (pentoxoresorufin-O-depentylation and 7-benzoyloxyquinoline-O-debenzylation) and immunoblotting (for Cyp2b10 and Cyp3a11).

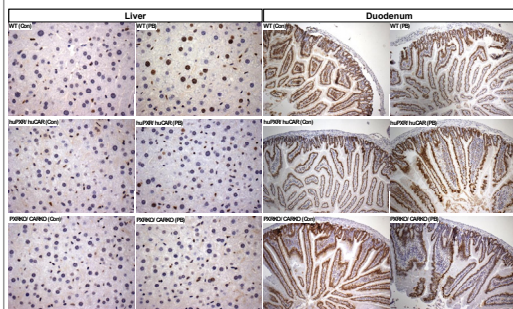
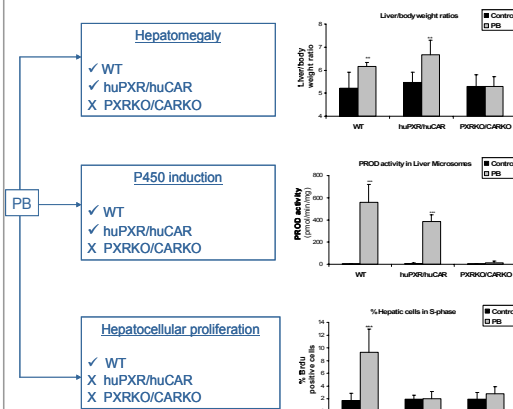
The nuclear incorporation of BrdU was determined as a measure of cell proliferation (S-phase). PB increased the hepatocellular labelling index (S-phase, % of cells labelled) by approximately 5-fold in the WT mice (WT control, 1.75 ± 1.10; WT PB-treated, 9.30 ± 3.64, P<0.001). However, no change in S-phase was detected in either the huPXR/huCAR (control, 1.93 ± 0.67; PB-treated, 2.00 ± 1.18) or PXRKO/CAR KO (control, 1.96 ± 1.04; PB-treated, 2.83 ± 1.07) mice following PB treatment.

## Fig 2: PB-mediated activation of Cyp3a11 and Cyp2b10 is predominantly CAR-dependent



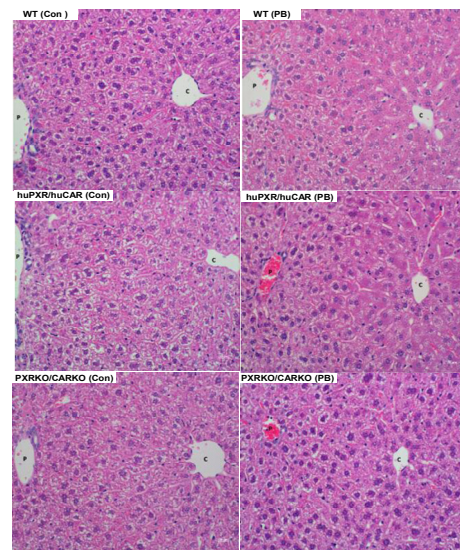
A panel of "humanised" and knockout PXR and CAR mouse lines have been generated and demonstrated to work in the predicted manner. Induction of Cyp2b10 and Cyp3a11 by PB (40 mg/kg ip/4 days) was ameliorated in PXR nulls, when compared with PB-treated WT mice, but not in animals that were devoid of CAR, as shown by immunoblot analysis and 7-benzoyloxyquinoline-O-debenzylation (BQ) and pentoxoresorufin (PROD) activities (n=3-5).

## Fig 3: hCAR does not support the hyperplasia response to PB



Treatment with PB (80 mg/kg/3 days) increased relative liver weights in the WT and huPXR/huCAR but not in the PXRKO/CAR KO mice (18% and 22%, respectively), as was Cyp2b10 induction (as determined by PROD activity, n=10). PB increased the S-phase in the WT mice by ~5-fold and appeared to have no effect on cell proliferation in the transgenic animals (data represents random counting of ~1.8 x 10<sup>5</sup> cells/group (n=10)). This WT mouse-specific hyperplasia was confirmed by immunohistochemistry (IHC) of BrdU-labelled hepatocyte nuclei (left panel, magnification, 400x). The presence of BrdU-labelled cells in duodenum served as a positive control for the IHC (right panel, magnification 100x).

## Fig 4: hCAR supports the hypertrophic response to PB



PB produced pathological evidence of centrilobular hepatocellular hypertrophy in WT and huPXR/huCAR but not the PXRKO/CAR KO mice, as determined by H&E analysis (magnification 200x). Mitotic figures indicating hepatocellular proliferation were identified only in PB-treated WT animals.

## Conclusions

• "Humanised" and knockout PXR and CAR mouse models have been successfully generated and characterised to investigate the mechanism of species differences in response to non-genotoxic carcinogens such as PB.

• Although PB is described as a PXR activator *in vitro*<sup>(4)</sup>, using the PXR/CAR mouse panel we have shown that PB-mediated activation of Cyp3a11 and Cyp2b10 is CAR-dependent, thus highlighting the limitations of *in vitro* data<sup>(2)</sup>.

• Following treatment with PB, P450 induction and hepatomegaly was observed in the WT and huPXR/huCAR mice but not in the PXRKO/CAR KO mice.

• Only WT and not huPXR/huCAR or PXRKO/CAR KO mice exhibited stimulated hepatocellular labelling index (S-phase) by PB.

• These data suggest that the human receptors are able to support the chemically induced hypertrophic response but not the hyperplastic response.

• These data demonstrate that the use of PXR/CAR panel is a powerful approach to investigating mechanisms of xenobiotic-induced cell regulation and proliferation and therefore is an essential tool in establishing the potential risk of rodent non-genotoxic carcinogens to man.

## References

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