

# A Novel Panel of Double "Humanised" & Knockout PXR and CAR Mouse Models

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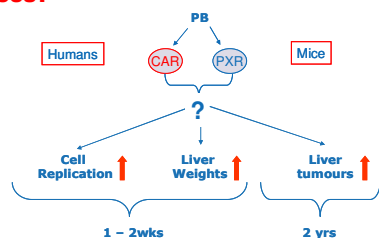


## Introduction

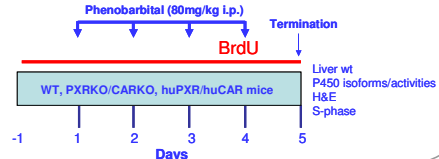
The interactions of chemicals with constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are complex, not only in the fact that they regulate common genes, but also because a single chemical agent can often interact with both receptors<sup>(1)</sup>. In order to fully understand the relative importance of the receptors in the efficacy and safety of drugs, we have created a novel panel of mouse models in which the murine genes have been exchanged for their human counterparts in the same locus (huPXR, huCAR) or knocked out (PXRKO, CARKO). In an attempt to resolve issues with receptor cross-talk and redundancy, the following models have also been created; huPXR/huCAR, PXRKO/CARKO, huPXR/CARKO and PXRKO/huCAR. Using this panel, "species-specific", CAR- and PXR-dependent differences have been demonstrated in the regulation of P450s, Phase II enzymes and transporters in response to treatment with xenobiotics.

CAR and PXR activators can also induce acute hepatomegaly to augment the ability of the liver to clear a xenobiotic stress. However, long-term treatments of rodents with these compounds can cause liver tumours, possibly due to their ability to increase cell proliferation and suppress apoptosis. The relevance of these tumours to human health is controversial due to the lack of a clear molecular mechanism and suitable human-like models. The clinically used drug, phenobarbital (PB) is a non-genotoxic carcinogen which has been shown to activate murine and possibly human CAR, however crosstalk with PXR may exist<sup>(2)</sup>. CAR knockout mice no longer activate the Cyp2b10 gene in response to PB, nor do the liver hypertrophic and hyperplastic responses elicited by these compounds occur<sup>(3-5)</sup>. Using this novel PXR/CAR mouse panel, we have investigated whether human CAR and PXR can mediate similar effects in the mouse. This would allow the evaluation of whether PXR and CAR mediate the effects of chronic xenobiotic stress on hepatocarcinogenesis in humans.

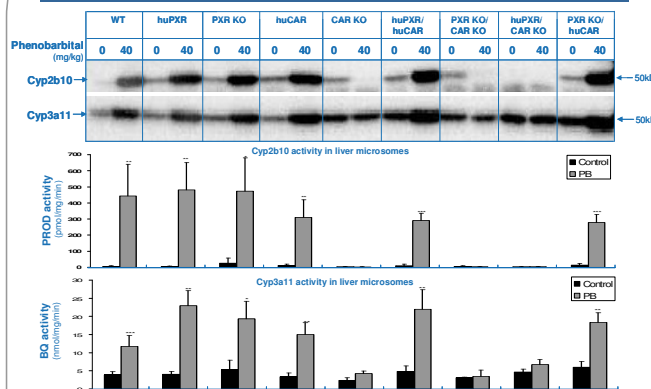
**Are hyperplastic responses to chemicals observed in animals relevant to humans and what are the molecular mechanisms behind these species differences?**



## Experimental Design

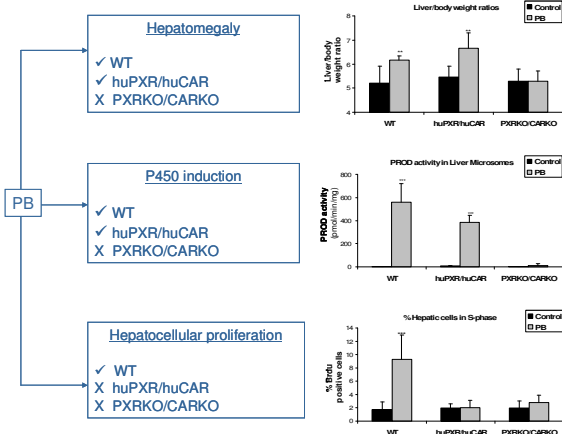


**Fig 1: PB-mediated activation of Cyp3a11 and Cyp2b10 is predominantly CAR-dependent**



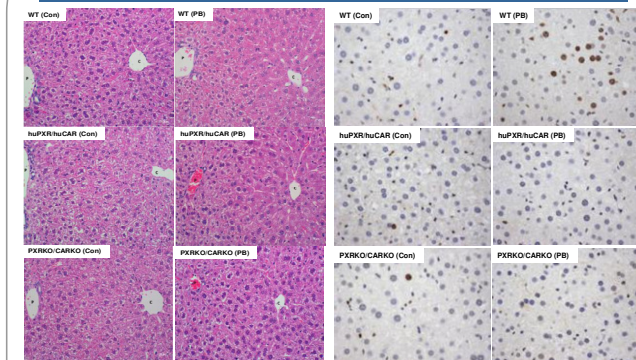
A panel of "humanised" and knockout PXR and CAR mouse lines have been generated and demonstrated to work in the predicted manner. Induction of Cyp2b10 and Cyp3a11 by PB (80mg/kg/3days) was ameliorated in PXR nulls, when compared with PB-treated WT mice, but not in animals that were devoid of CAR, as shown by immunoblot analysis and benzyloxyresorufin-O-demethylase (BO) and pentoxyresorufin (PROD) activities (n=3-5).

**Fig 2: hCAR does not support the hyperplasia response to PB**



Treatment with PB (80mg/kg/3days) increased relative liver weights in the WT and huPXR/huCAR but not in the PXRKO/CARKO 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)).

**Fig 3: hCAR supports the hypertrophic response to PB**



PB produced pathological evidence of centrilobular hepatocellular hypertrophy in WT and huPXR/huCAR but not the PXRKO/CARKO mice, as determined by H&E analysis (left panel, magnification 200x). Mitotic figures indicating hepatocellular proliferation were identified only in PB-treated WT animals. This WT mouse-specific hyperplasia was further confirmed by immunohistochemistry of BrdU-labelled hepatocyte nuclei (right panel, magnification, 400x).

## Conclusions

- We have successfully generated a panel of functional "humanised" and knockout PXR and CAR 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>(6)</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.
- Following treatment with PB, P450 induction and hepatomegaly was observed in the WT and huPXR/huCAR mice but not in the PXRKO/CARKO mice.
- Only WT and not huPXR/huCAR or PXRKO/CARKO 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.
- We have demonstrated 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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