

A Novel CYP3A Cluster Humanised Mouse Model For Improved Prediction of Human Response

J. Ross¹, Y. Kapelyukh¹, N. Scheer², A. Rode² and C.R. Wolf¹.

CXR Biosciences Ltd, Dundee, Scotland¹ and TaconicArtemis, Koln, Germany².

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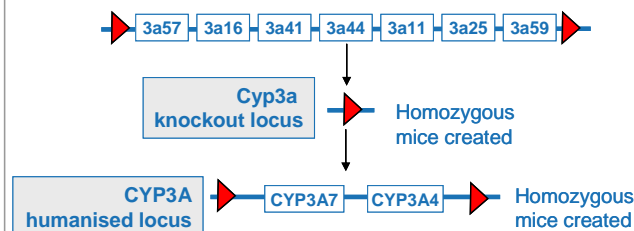
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Introduction

The cytochrome P450 isoenzyme CYP3A4 is one of the major enzymes responsible for the metabolism of xenobiotics in humans. In order to improve the prediction of human metabolism from animal studies, we have developed a novel humanised mouse model for CYP3A in which the murine genes from the Cyp3a subfamily were replaced with two of their human counterparts by introducing CYP3A4 and CYP3A7 into the Cyp3a locus (termed hCYP3A4/3A7_Cyp3a KO). An equivalent knockout for Cyp3a isoforms was also created (termed Cyp3a KO).

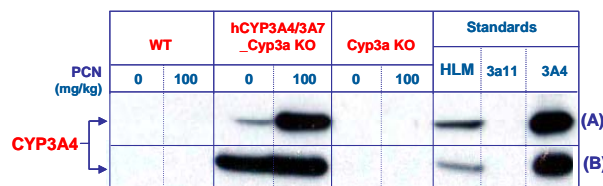
Using RNA and protein analysis, we demonstrated that CYP3A4 was expressed constitutively in the liver and small intestine of hCYP3A4/CYP3A7_Cyp3a KO mice and was highly induced by treatment with 5-pregnen-3 β -ol-20-one-16 α -carbonitrile (PCN), a potent inducer of CYP3A4 expression. Interestingly, CYP3A7 RNA was also found in the liver of PCN-treated animals whilst constitutive CYP3A7 RNA expression was below the limit of detection. Induction with PCN resulted in a significant increase in the rate of oxidation of CYP3A4-specific substrates by liver and intestinal microsomes from hCYP3A4/CYP3A7_Cyp3a KO mice.

Fig 1: Targeting strategy for hCYP3A4/3A7_Cyp3a KO mouse



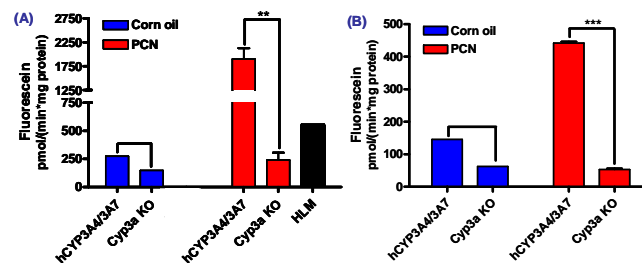
- Created using a CYP3A4/3A7 BAC
- Under the control of human CYP3A4 and CYP3A7 promoters
- Human genes and/or gene clusters targeted to mouse locus
- Both lines on the same genetic background (C57BL/6J)

Fig 2: Basal CYP3A4 levels comparable to those seen in male human liver



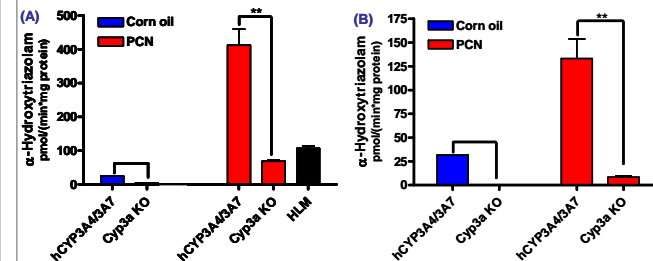
CYP3A4 protein expression in (A) liver and (B) intestinal microsomes from WT, hCYP3A4/3A7_Cyp3a KO and Cyp3a KO mice. Male mice were treated with either corn oil or PCN (100 mg/kg/2 days/IP). Liver and intestinal microsomes (10 μ g, respectively) were loaded onto SDS-PAGE gels. Blots were incubated in a polyclonal rabbit anti-CYP3A4 (Gentest, cat # 458234). Standards: HLM - pooled male human liver microsomes (10 μ g) (Gentest, cat # 452172); 3a11 - murine Cyp3a11 recombinant protein (0.1 pmol) (Dr. Henderson, Uni. of Dundee, UK); 3A4 - human CYP3A4 baculosomes (0.1 pmol) (Invitrogen, cat # P2377).

Fig 3: Catalytically active hepatic and intestinal CYP3A4



Dibenzylfluorescein (DBF) oxidation by (A) liver and (B) intestinal microsomes from hCYP3A4/3A7_Cyp3a KO and Cyp3a KO mice. In both liver and intestinal microsomes from the humanised mice, DBF oxidation was increased compared to Cyp3a KO mice. Following PCN treatment, the reaction rate was markedly higher in hCYP3A4/3A7_Cyp3a KO mice than in Cyp3a KO mice. DBF incubations consisted of; 2 μ M DBF, 5 μ g microsomes, 50 mM HEPES buffer pH7.4 and 42 mg/mL NADPH at 37°C for 50 secs. Fluorescence was measured using an F-4500 fluorescence spectrophotometer. Values represent mean \pm SD (n=3; PCN-treated mice) and data from individual control mice. Data from hCYP3A4/3A7_Cyp3a KO mice were significantly different when compared to Cyp3a KO mice by; **P<0.01; ***P<0.001 (paired Student's T-test).

Fig 4: Impact of CYP3A4 on Triazolam Metabolism



Triazolam oxidation by (A) liver and (B) intestinal microsomes from hCYP3A4/3A7_Cyp3a KO and Cyp3a KO mice. Significantly higher activity was detected in hCYP3A4/3A7_Cyp3a KO mouse microsomes when compared with Cyp3a KO mice. Triazolam (50 μ M) was incubated with 2.5 μ g microsomes, 50 mM HEPES buffer pH7.4 and 1.3 mM NADPH at 37°C for 15 mins then α -hydroxytriazolam concentration was determined by LC-MS/MS. Values represent mean \pm SD (n=3; PCN-treated mice) and data from individual control mice. Data from hCYP3A4/3A7_Cyp3a KO mice were significantly different when compared to Cyp3a KO mice; **P<0.01 (paired Student's T-Test).

Conclusions

- hCYP3A4/3A7_Cyp3a KO mice demonstrated constitutive expression of hepatic CYP3A4 protein expression comparable to those levels seen in pooled human liver microsomes.
- A full-length CYP3A4 transcript specific to the humanised mouse was detected, cloned and verified by sequence analysis (data not shown).
- Marked induced CYP3A4 expression was observed in hCYP3A4/3A7_Cyp3a KO mice in response to PCN treatment. Intestinal CYP3A4 protein was readily detected in hCYP3A4/3A7_Cyp3a KO mice.
- Oxidation of the CYP3A4 specific substrates DBF and triazolam was significantly increased in the humanised mice when compared to Cyp3a KO mice, indicating that CYP3A4 is catalytically active.

We have demonstrated a fully functional humanised CYP3A mouse model which can be used, in combination with the Cyp3a KO model, as tools:

- To identify the *in vivo* effects of human metabolites
- For improved CYP induction and inhibition screening
- To elucidate the *in vivo* effects of metabolism on bioavailability

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