CYP3A4 and Pregnane X Receptor Humanized Mice

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ABSTRACT: Marked species differences exist in P450 expression and activities. In order to produce mouse models that can be used to more accurately predict human drug and carcinogen metabolism, P450- and xenobiotic receptor humanized mice are being prepared using bacterial artificial chromosomes (BAC) and P1 phage artificial chromosomes (PAC) genomic clones. In some cases, transgenic mice carrying the human genes are bred with null-mice to produce fully humanized mice. Mice expressing human CYP1A1, CYP1A2, CYP2E1, CYP2D6, CYP3A4, and CYP3A7 were generated and characterized. Studies with the CYP3A4-humanized (hCYP3A4) mouse line revealed new information on the physiological function of this P450 and its role in drug metabolism in vivo. With this mouse line, CYP3A4, under certain circumstances, was found to alter the serum levels of estrogen resulting in deficient lactation and low pup survival as a result of underdeveloped mammary glands. This hCYP3A4 mouse established the importance of intestinal CYP3A4 in the pharmacokinetics of orally administered drugs. The hCYP3A4 mice were also used to establish the mechanisms of potential gender differences in CYP3A4 expression (adult female > adult male) that could account for human gender differences in drug metabolism and response. The pregnane X receptor (PXR) is also involved in induction of drug metabolism through its target genes including CYP3A4. Since species differences exist in ligand specificity between human and mice, a *PXR*-humanized mouse (hPXR) was produced that responds to human PXR activators such as rifampicin but does not respond to the rodent activator pregnenalone 16α-carbonitrile. © 2007 Wiley Periodicals, Inc. J Biochem Mol Toxicol 21:158–162, 2007; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10:1002/jbt.20173

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HUMANIZED MICE

Because of the marked species differences in P450 expression, there are no reliable rodent models that can be used to determine the metabolism of drugs in vivo. In order to rectify this problem, P450-humanized mice were developed. Humanized mice are produced by transgenesis using two distinct strategies. The P450 cDNA can be placed behind a promoter in an expression vector and introduced into mice by the standard pronuclei injection methodology. In most cases, a liver-specific promoter, such as the rat serum albumin promoter or the mouse albumin (transthyretin) promoter, is used to deliver liver hepatocyte-specific expression of protein. Another means to introduced human proteins into mice is to deliver the whole human gene including all exons, introns, and regulatory elements. This can be accomplished by use of genomic clones derived from λ phage, bacterial artificial chromosomes (BAC), or P1 phage artificial chromosomes (PAC). The genes are under control of their own regulatory elements, and their human tissue-specific regulation and induction patterns should be maintained in the mouse assuming that most transcription factors that control gene expression are conserved in mammals. Ideally, the transgenes should be introduced onto a null mouse background. However, practically, where the corresponding mouse gene locus is a large family extending over hundreds of base pairs, a null mouse may not be available.

Several P450-humanized mice have been produced. Mice expressing the polymorphic CYP2D6 were made using a λ phage genomic clone. These animals were able to metabolize the debrisoquine to the 4OH-debrisoquine metabolite found in humans given the drug, and thus would be equivalent to human poor metabolizers of debrisoquine [1]; wild-type mice do not metabolize the drug. Mice humanized for CYP1A1 and CYP1A2 were made [2,3]. This animal model was used to demonstrate an important species difference in the metabolic activation of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Mouse CYP1A2 preferentially produces the inactive 4′OH-PhIP, whereas human CYP1A2 produced the N^2OH

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derivative of PhIP that leads to the active metabolite capable of binding to DNA [2,4]. Mice humanized for CYP2E1 exhibited altered sensitivity to the hepatotoxicity of acetaminophen [5]. These data with CYP1A2 and CYP2E1 suggest that humanized mice might be more appropriate to determine human risk assessment to chemical exposures than wild-type mice.

CYP3A4-HUMANIZED MICE

CYP3A4 is the most abundant P450 expressed in human livers and metabolizes more than 50% of clinically used drugs. CYP3A4 is also expressed in the gut, where it can metabolize orally administered drugs. Since this P450 metabolizes many clinically used drugs, it is involved in drug–drug interaction, where one compound inhibits the metabolism of another agent. With drugs on the market, the drug insert usually states whether the compound is metabolized by CYP3A4 in order to avoid co-administration of two CYP3A4 substrates. During preclinical development, drug candidates can be screened using recombinant P450s to determine whether a compound is metabolized by CYP3A4. However, in vivo models to study CYP3A4 have not been available. Mice have negligible CYP3A P450s in the liver and gut unless they are treated with inducers that activate the pregnane X receptor (PXR) or constitutive androstane receptor (CAR).

In an effort to produce a CYP3A4-humanized mouse line, a BAC clone (Figure 1A) was used that contained the complete CYP3A4 gene including the upstream PXR-binding sites [6]. This mouse line (hCYP3A4) expressed CYP3A4 in the small intestine of the transgenic mouse, and expression was not observed in the small intestine of wild-type mice (Figure 1B). However surprisingly, little expression of CYP3A4 was detected in the liver in the absence of administration of an inducer such as the PXR ligand pregnenolone 16α-carbonitrile or the CAR activator phenobarbital. Levels of expression in the hCYP3A4 gut were comparable to that found in human gut microsomes. This expression of CYP3A4 in gut influenced the metabolism and pharmacokinetics of orally administered as drugs and was demonstrated by the higher rate of elimination of the CYP3A4 probe drug substrate midazolam as compared to non-CYP3A4 expressing wild-type mice [6]. No difference in midazolam pharmacokinetics was observed when the drug was administered intravenously. This mouse model was used to demonstrate a possible drug–drug interaction between midazolam and the potent CYP3A4 inhibitor ketaconozole. Oral administration of midazolam and ketaconozole resulted in a markedly lower rate of elimination of midazolam and compared to midazolam administered with the inhibitor (Figure 2).

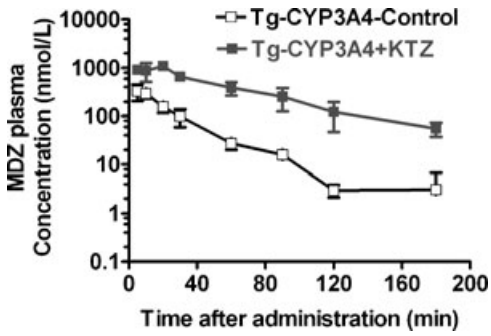

FIGURE 2. Pharmacokinetics of midazolam (MDZ) in hCYP3A4 mice orally administered MDZ in the absence and presence of the CYP3A4 inhibitor ketaconozole.

The finding of low expression of CYP3A4 in the livers of hCYP3A4 mice was initially puzzling. However, the earlier studies were done with mature male mice. Later studies revealed that immature male and adult female mice expressed the enzyme in the liver indicating the transgene is developmentally regulated in a gender-specific manner [7]. In both males and females, CYP3A4 protein is expressed as early as 1 week

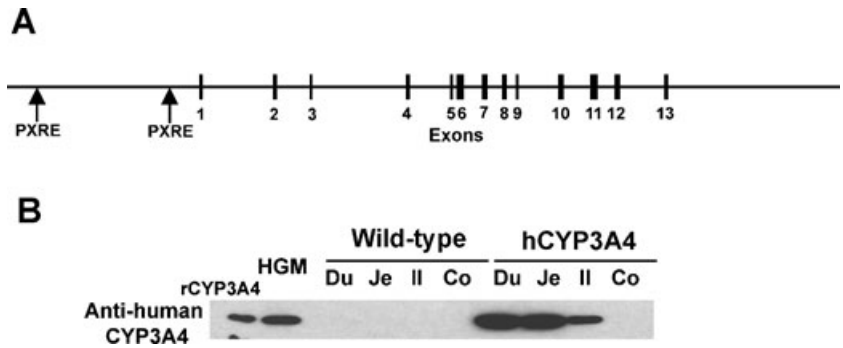

FIGURE 1. Structure of the CYP3A4 transgene used to produce the hCYP3A4 mouse line (A). Western blot of CYP3A4 expression in human gut microsomes (HGM), duodenum, (Du), jejunum (Je), ileum (Il), and colon (Co) of wild-type and hCYP3A4 mice (B).

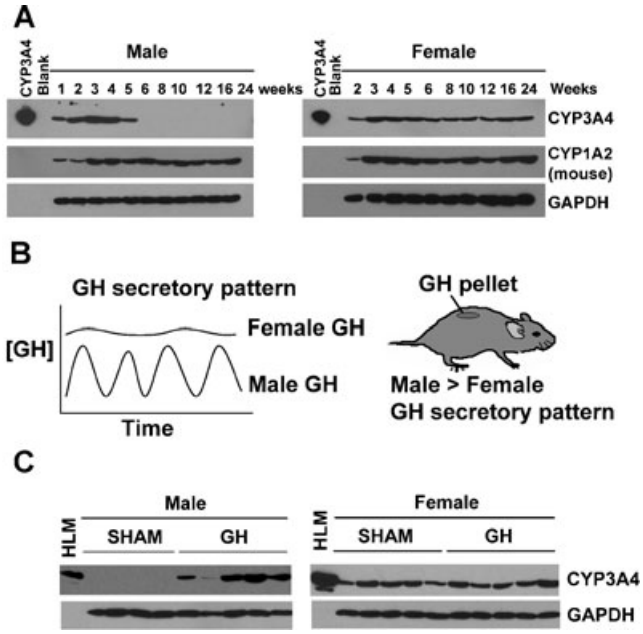

FIGURE 3. Gender-dependent regulation of constitutive expression of CYP3A4 by growth hormone. Developmental expression of CYP3A4 protein in livers of male and female mice (A). Antibodies against CYP1A2 and GAPDH were used as controls. Depiction of secretory patterns of growth hormone in male and female mice and implantation of growth hormone-containing pellet to mice to convert the male secretory pattern to the female pattern (B). Expression of CYP3A4 protein in male and female mice implanted with a growth hormone pellet (C). GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GH: growth hormone; SHAM: implanted with an empty pellet.

after birth. In 6-week-old male mice, expression is extinguished at 6 weeks of age but remains in females (Figure 3A). In contrast, expression of the *Cyp1a2* gene is similar in both genders throughout early development. Gender-specific regulation of P450s has been described in rodent model systems and is due to the differences in growth hormone secretory patterns between males and females [8]. Female mice secrete high constant levels of growth hormone, whereas males secrete lower levels of the hormone in a cyclical manner. The male growth hormone secretory pattern can be converted to the female pattern by implantation of growth hormone pellets (Figure 3B). When this is done, 6-week-old male mice express CYP3A4 (Figure 3C). These data demonstrate that CYP3A4 is under control by the growth hormone signal transduction pathway in the liver. This is in agreement with human studies indicating a higher expression of CYP3A4 in females [9].

Another interesting finding with one line of hCYP3A4 mice was the low-estradiol levels in the serum of pregnant dams that resulted in underdeveloped mammary glands, low-milk production, and low-pup survival growth rate due to their inability to nurse [10]. The low-serum estrogen is likely the result of increases in metabolism of estradiol to inactive 2- and 4-hydroxylated estradiol metabolites in the gut by CYP3A4 [12]. The clinical implications of this finding are not known but needs to be investigated.

Note that the mouse *Cyp3a* genes have not been disrupted, and thus the hCYP3A4 mouse described above have a mouse CYP3A background. In order to produce a fully humanized mouse line, the *Cyp3a* locus needs to be disrupted. This represents a challenge since there are four active mouse genes (*Cyp3a11*. *Cyp2a13*, *Cyp3a25*, *Cyp3a41*) and a number of pseudogenes located on about 8 Mb of DNA. The *Cyp3a11, Cyp2a13*, and *Cyp3a25* genes are expressed in the liver and activated by ligands to PXR [12]. Because of the proximity of the genes, they must all be disrupted from the same chromosome, a daunting task that requires use of the Cre-loxP system [13].

PXR-HUMANIZED MICE

PXR is the main nuclear receptor responsible for regulation of the CYP3A P450s. It is activated by a large number of xenobiotics including many clinically used drugs. However, species differences occur in PXR ligand specificity between humans and mice [14]. For example, pregnenalone 16α-carbonitrile (PCN) activates the mouse PXR but does not activate human PXR whereas rifampicin (RIF) preferentially activates human PXR. Thus, to study the in vivo effects of human PXR, a humanized mouse line was developed by placing the human PXR cDNA behind a liver-specific promoter and making a transgenic mouse with the construct and then breeding the transgene into a *Pxr*-null mouse background [15]. In contrast to a wild-type mouse, this line displayed induction of target genes by the human PXR ligand rifampicin. However, in this model, the human PXR is only expressed in the liver and not other tissues, where the receptor could impact xenobiotics metabolism.

More recently, a PXR humanized mouse line was produced using a similar strategy except that the complete human PXR gene under control of its native promoter was used [16]. A BAC clone was injected to make a transgenic mouse that was then bred with Pxr-null mice to generate a fully humanized hPXR mouse (Figure 4A). The human PXR gene was expressed in the small intestine and liver, the major sites of CYP3A expression and regulation by PXR in these species (Figure 4B). To monitor activation of PXR by PCN and rifampicin in wild-type and hPXR mice, the mouse CYP3A11 mRNA was monitored. The CYP3A11 mRNA was marked induced by PCN in wild-type mice but not in Pxr-null mice or hPCN mice (Figure 4C). In contrast, rifampicin did not induce CYP3A11 mRNA

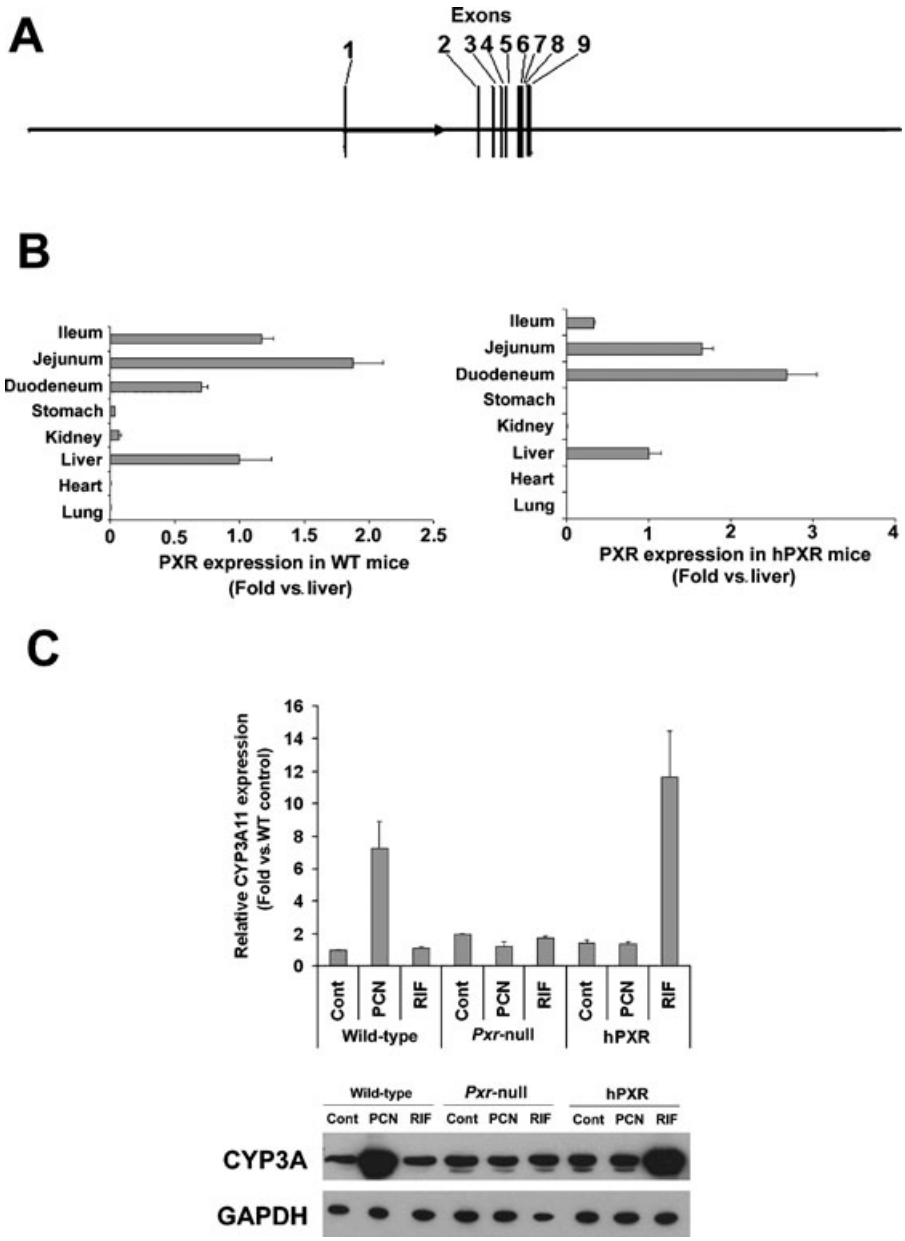

FIGURE 4. Structure of the PXR transgene used to produce the hCYP3A4 mouse line (A). Expression of mouse and (left) and human (right) PXR mRNA in different tissues of wild-type and hPXR mice as measured by real-time quantitative PXR (B). Induction of CYP3A11 mRNA by PCR and rifampicin (RIF) in wild-type, *Pxr*-null and hPXR mice (C).

in wild-type or *Pxr*-null mice but readily induced the mRNA in hPXR mice. These data demonstrate that hPXR mice can be used to evaluate human PXR activators in a whole animal model.

CONCLUSIONS AND FUTURE PERSPECTIVES

The studies described above show that P450 and xenobiotic receptor humanized mice can be produced using whole human genes introduced into mice with BAC or PAC clones. The mice have proven to be very stable with some lines having been maintained for several years without loss of expression of the transgene. These mice will be of value for study of the role of P450s in the gut in the pharmacokinetics or orally administered drugs and in the mechanism of regulation of human P450 genes. Future studies include the generation of a CYP3A4 humanized mouse line that lacks the endogenous mouse genes. It is also possible to make a CYP3A4 and PXR double

humanized mouse line that could be used in drug development.

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