Genomic characterization and regulation of CYP3a13: role of xenobiotics and nuclear receptors


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SPECIFIC AIMS

Cytochromes P450 (CYPs) have been demonstrated to catalyze phase I metabolism of various drugs in clinical use. The CYP3A subfamily is responsible for metabolizing >50% of all drugs. CYP3A isoforms show a gender-, tissue-, and age-dependent expression pattern. This makes the CYP3A isoforms interesting because, apart from drug–drug interactions, CYP3A isoforms may play an important role in the differential drug efficacy observed in males and females. The purpose of this study was to characterize the genomic structure of mouse CYP3a13 in order to identify the molecular basis of CYP3a regulation and test the catalytic ability of expressed CYP3a13 toward clinically active drugs. Since various nuclear receptors can differentially regulate CYP3a isoforms, we examined the in vivo role of pregnane X receptor (PXR), retinoid X receptor (RXR), and peroxisome proliferator-activated receptor gamma (PPARγ) ligands in mediating CYP3a13 expression.

PRINCIPAL FINDINGS

1. Genomic structure characterization

Using 5′ rapid amplification of cDNA ends by PCR (5′RACE), we determined the transcriptional initiation site of CYP3a13 gene. The genomic structure of mouse CYP3a13 closely resembles human CYP3A43. CYP3a13 gene is localized on mouse chromosome 5. The gene organization reveals that CYP3a13 gene spans 27.5 kb and contains 13 exons ranging from 53 to 366 bp. The transcription start site as determined is located 35 bp upstream of the first nucleotide of cDNA and results in 109 bp of 5′ untranslated region. Putative promoter elements in the 5′ flanking region of CYP3a13 were identified using the TRANSFAC program. The search engine revealed several RXR half sites as well as PXR and estrogen receptor binding sites. No TATA box was observed up to as far as 1.3 kb away from transcription start site; however, SP1 and CAAT binding sites were located near the start site.

2. Tissue distribution and sexual dimorphic expression

CYP3a13 mRNA expression was quantified using the Quantitative Real-Time PCR (QRTPCR) assay. Using this assay we were able to detect CYP3a13 in extrahepatic tissues: lung, kidney, and brain. The tissue distribution of CYP3a13 can be summarized as liver > lung > brain > kidney.

Despite being an ortholog of female-specific rat CYP3A9, CYP3a13 did not exhibit sexual dimorphic expression. We compared CYP3a13 expression in controls, ovariectomized, and ovariectomized mice injected with estradiol benzoate (500 μg/kg/animal/day) for a week. We did not observe any significant change in the level of expression among these groups.

3. RXR and PPARγ ligands inhibit CYP3a13 expression

RXR ligand LG268 (30 mg/kg) or PPARγ ligand rosiglitazone (30 mg/kg) treatment of C57Blks/J mice for 2 wk caused down-regulation of CYP3a13 mRNA expression. CYP3a13 expression was significantly decreased by a maximum of 50% in liver and 80% in brain after LG268 treatment while no change of expression was observed in the kidney (Fig. 1A). The PPARγ ligand decreased CYP3a13 expression in liver by 50% but not in brain or kidney (Fig. 1B). Thus, RXR and PPARγ ligands can down-regulate CYP3a13 gene expression in a tissue-specific manner.

4. PXR regulates CYP3a13 basal levels, and dexamethasone (DEX) induction of CYP3a13 is observed only in PXR null mice

To investigate the role of PXR in hepatic induction of CYP3a13, wild-type and PXR−/− mice were treated with...
We found a marked decrease in CYP3a13 expression in the absence of PXR (Fig. 2A). Thus, PXR plays an important role in basal expression of CYP3a13 in contrast to mouse CYP3a11. DEX treatment (100 mg/kg) once daily for 4 days did not lead to a significant induction of CYP3a13 in wild-type mice. DEX treatment of PXR null mice significantly induced CYP3a13 expression and restored the levels to that of the wild-type (Fig. 2A). On the contrary, CYP3a11 showed marked induction by DEX in wild-type mice, which was completely absent in PXR null mice (Fig. 2B).

**5. Recombinant expressed CYP3a13 protein can catalyze N-demethylation of benzphetamine, ethylmorphine, and erythromycin**

To test the catalytic profile of CYP3a13 toward some clinically used drugs, we subcloned CYP3a13 in pCMV-Script expression vector and transfected it into COS M6 cells. CYP3a13 protein expression was checked by protein immunoblot using rat polyclonal CYP3A2 antibody. The N-demethylation activity toward benzphetamine, ethylmorphine, and erythromycin was determined using control and CYP3a13-expressing COS cell lysates. The specific activity values observed for benzphetamine, ethylmorphine, and erythromycin were 23.62 ± 0.82, 15.22 ± 0.34, and 18.38 ± 0.2, respectively.

**CONCLUSIONS AND SIGNIFICANCE**

We found that although mouse CYP3a13 shows maximum homology to the female-specific rat CYP3A9, CYP3a13 expression is not sexually dimorphic. Apart from predominant liver expression, CYP3a13 is transcribed in lung, brain, and kidney. The functional significance of this extrahepatic expression needs further characterization; however, this tissue distribution is consistent with tissue-specific expression of the nuclear receptors (e.g., PXR) involved in CYP3A gene regulation.

CYP3a13 gene is located on mouse chromosome 5, spans 27.5 kb, and contains 13 exons. No TATA box was observed up to 1.3 kb upstream of the start site, but a CAAT box was identified in the region close to the transcription initiation site. We also found an SP1 binding site near the CAAT box. TATA-less promoters...
for CYP genes are not uncommon. It is known that SP1 sites are crucial in transcription initiation for many TATA-less promoters.

Although CYP3A genes are known to be induced by glucocorticoids, CYP3a13 sequence analysis failed to identify any glucocorticoid receptor (GR) response element. To test whether the GR plays any role in CYP3a13 induction, we evaluated CYP3a13 mRNA expression after treatment with DEX, a well-characterized ligand for GR. The results clearly indicate that CYP3a13 is not induced by DEX in wild-type mice; however, DEX treatment can significantly induce CYP3a13 expression only in PXR null mice. Based on these findings, we speculate that there are additional players or compensatory mechanisms other than PXR involved in mediating CYP3a13 gene expression.

We found that RXR and PPARγ ligands can down-regulate CYP3a13 gene expression in a tissue-specific manner. As previously reported, the livers of mice treated with RXR and PPARγ ligand exhibited hepato-megaly and a spongy appearance. These observations illustrate the intricate signaling between different nuclear receptors and CYP3a isoforms to maintain cellular homeostasis.

In this study we observed that CYP3a13 recombinant-expressed protein was effective in catalyzing N-demethylation of benzphetamine, ethylmorphine, and erythromycin. These results indicate that expressed CYP3a13 has drug metabolizing capabilities and that its specific activities are comparable to those observed for rat CYP3A isoforms. These findings open insights into nuclear receptor-mediated pathways through which CYP3As can be regulated (Fig. 3). This may provide a mechanism for clinically observed drug–drug interactions. Our observation that different nuclear receptors regulate CYP3a13 differentially may have significant impact in the clinical scenario as many of the newly synthesized ligands targeted toward these nuclear receptors could influence CYP3A gene expression.

**Figure 3.** A schematic diagram presenting the putative regulation of CYP3a13 gene expression and catalytic activity. This schematic depicts the genomic structure of CYP3a13, consisting of 13 exons in stick figure format. CYP3a13 promoter is TATA-less but contains several SP1 sites. Upon ligand binding, nuclear receptors dimerize and translocate to the nucleus, where they bind their corresponding response elements on the DNA leading to regulation of gene expression of certain genes. Activation of RXR by LG268 or PPARγ by rosiglitazone leads to significant down-regulation of CYP3a13 gene expression. CYP3a13 protein is membrane bound in endoplasmic reticulum (ER), where it can catalyze the metabolism of CYP3A substrates. B) CYP3a13 gene regulation by the xenobiotic nuclear receptor PXR has been implicated as a key regulator of CYP3a gene expression. While basal levels of CYP3a13 are markedly decreased in PXR−/− mice (lack DNA binding domain (DBD)) compared with the wild-type, DEX can induce CYP3a13 expression only in PXR null mice. Based on these findings, we speculate that there are additional players or compensatory mechanisms other than PXR involved in mediating CYP3a13 gene expression.