A TRANSGENIC MOUSE MODEL WITH A LUCIFERASE REPORTER FOR STUDYING IN VIVO TRANSCRIPTIONAL REGULATION OF THE HUMAN CYP3A4 GENE

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(Received February 14, 2003; accepted May 1, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Cytochrome P450 3A4 (CYP3A4) plays an important role in drug metabolism, and the enzymatic activity of CYP3A4 contributes to many adverse drug-drug interactions. Here we describe a transgenic mouse model that is useful in monitoring the in vivo transcriptional regulation of the human CYP3A4 gene. A reporter construct consisting of 13 kilobases of the human CYP3A4 promoter controlling the firefly luciferase gene was used to generate a transgenic mouse line [FVB/N-Tg(CYP3A4-luc)Xen]. Reporter gene expression was assessed using an in vivo imaging system (IVIS) in anesthetized mice. Basal expression of the reporter was highest in liver and kidney, and moderate in the duodenum in male transgenic mice, whereas the basal luciferase activity was highest in the duodenum and lower in kidney and liver in females. Injections of pregnenolone, phenobarbital, rifampicin, nifedipine, dexamethasone, 5-pregnen-3ß-ol-20-one-16ß-carbonitrile (PCN), and clotrimazole resulted in a time-dependent induction of luciferase expression, primarily in liver, that peaked at 6 h post injection. The greatest induction was found with clotrimazole, dexamethasone, and PCN, whereas the lowest induction followed pregnenolone, phenobarbital, and rifampicin injection. In general, male mice responded to these drugs more strongly than did females. Our results suggest that the human CYP3A4 promoter functions in transgenic mice and that this in vivo model can be used to study transcriptional regulation of the CYP3A4 gene.
Cyp3a protein at a dose of 50 to 100 mg/kg body weight (Yanagimoto et al., 1997; Schuetz et al., 2000), whereas rifampicin did not activate mouse PXR (mPXR) (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998). The ultimate goal of these in vitro assays is to predict the in vivo response, and significant efforts have been made to generate predictive models (Schmider et al., 1999; Houston and Kenworthy, 2000). However, the ability of in vitro assays to reflect in vivo response is complicated by factors such as the influence of route of administration on drug distribution, metabolism by multiple organs, dosing, and temporal effects (Thummel and Wilkinson, 1998). Although many in vivo animal models have proven to be beneficial for assessing P450 induction, measurements of mRNA, protein, or activity of CYP3As involve many time-consuming steps such as tissue sampling, RNA and protein extractions, and Northern and Western analyses. More efficient in vivo models are needed to evaluate the effects of compounds on P450 metabolism and induction.

To address the unmet need for efficient in vivo models for studying CYP3A4 regulation, we developed a transgenic mouse model consisting of 13 kb of the human CYP3A4 promoter controlling expression of the firefly luciferase gene. This reporter system is similar to an in vivo reporter described by Schuetz et al. (2002) and an in vitro reporter described by Goodwin et al. (1999). Regulation of the reporter gene in whole animals in response to seven CYP3A4 inducers was rapidly assessed using an in vivo imaging system (IVIS) (Contag et al., 1995). This approach of “biophotonic” imaging allows the noninvasive study of the time course of a response using a highly light-sensitive camera system. Our data suggest that the human CYP3A4 promoter functions in transgenic mice and that this model could be used to study transcriptional regulation of the CYP3A4 gene and CYP3A4-mediated drug-drug interactions in vivo.

Materials and Methods

Generation of the CYP3A4-luc Transgenic Line. A bacterial artificial chromosome clone containing the human CYP3A4 promoter region was screened by PCR using primers 5′-GGTGTTACCTGCACTGACACTGTCGCCCATCATG-3′ corresponding to nucleotides −1105 to −1080 and 5′-ATCAAGCTTCTTACGCTGTCTCTCTTC-3′ corresponding to nucleotides −40 to 69 of the CYP3A4 promoter region (Incyte Genomics Inc., Palo Alto, CA). The primers were also used to amplify a 1.2-kb reporter region of CYP3A4 from human genomic DNA using pfu DNA polymerase (Stratagene, La Jolla, CA). The PCR product was digested with KpnI/HindIII and purified from an agarose gel using a GeneClean Kit (Bio 101, Vista, CA). The 1.2-kb promoter region was cloned into the pGL3-Basic vector (Promega, Madison, WI). A 233-bp HindIII fragment containing a chimeric intron from pCAT-3-Basic vector (Promega) was then inserted between the CYP3A4 promoter region and the luciferase gene. A 1.88-kb KpnI/BglII fragment, a 950-bp BglII fragment, and a 10-kb KpnI fragment subcloned from the bacterial artificial chromosome clone were inserted sequentially into the above construct. The pGL3-1.3A4 plasmid was digested with Sool, and a 15-kb DNA fragment containing a 13-kb CYP3A4 promoter, the chimeric intron, and the firefly luciferase cDNA was purified from agarose gel by electrophoresis (Fig. 1A). The purified fragment was then microinjected into single-cell-stage FVB/N embryos, and the embryos were implanted into pseudopregnant mice.

Genotyping of CYP3A4-luc Transgene. PCR Assay. Transgenic founders and offspring were identified by PCR using luciferase primers Luc F (5′-GAAGATTCGCGGTTGCTGGCAGAACG-3′) and Luc R (5′-CCTAACACTGTATGGAAGTGAACAAAC-3′).

Southern Blotting. Mouse genomic DNA was isolated from mouse tail tips using the DNeasy Tissue Kit (QIAGEN, Valencia, CA). Mouse genomic DNA was digested with the BamHI restriction enzyme and DNA fragments were resolved on a 1% agarose gel. DNA was treated with 250 mM HCl for 10 min and denatured in 20X standard saline citrate with 0.5 M NaOH for 10 min, and then transferred onto Hybond N+ membrane (Amersham Biosciences Inc., Piscataway, NJ) with 20X standard saline citrate containing 0.5 M NaOH for 1–2 h using a vacuum blotting apparatus (Stratagene). After transferring, DNA was fixed to the membrane using a UV Cross-Linker (Stratagene). A 1.8-kb Ncol/BglII fragment from pGL3-Basic (Promega) containing the entire luciferase cDNA was isolated from an agarose gel and used as a probe. Luciferase DNA probe (100 ng) was labeled using AlkPhos Direct Labeling and detected using the Detection System with CDP-Star (Amersham Biosciences Inc.).

Ex Vivo Luciferase Assay. Liver, duodenum, kidney, spleen, lung, heart, and offspring were identified by PCR using luciferase primers Luc F (5′-GAAGATTCGCGGTTGCTGGCAGAACG-3′) and Luc R (5′-CCTAACACTGTATGGAAGTGAACAAAC-3′).

Screening CYP3A4-luc Founders. For primary screening of each transgenic line, a group of three mice including both genders (one male and two females or two males and one female) were imaged before injection (T = 0) and then injected i.p. with 100 μl of dimethyl sulfoxide (DMSO), or dexamethasone or rifampicin at 50 mg/kg body weight. Mice were imaged, as described below, at 3 and 6 h after injection. The criteria used for screening were: 1) relatively high basal luciferase expression in liver and 2) up-regulation of luciferase expression in liver both dexamethasone and rifampicin injection. Nine lines were screened and one line with the best characteristics was selected for characterization.

Animal Studies. Induction of the CYP3A4-luc transgene by seven drugs. Male and female CYP3A4-luc transgenic mice at 6 to 8 weeks of age, in groups of three, were injected i.p. with 100 μl of DMSO, or dexamethasone, rifampicin, pregnenolone, nifedipine, 5-pregnen-3,16α-diol-20-one-16α-carbonitrile (PCN), or clotrimazole dissolved in DMSO at 50 mg/kg body weight. Phenobarbital was administered at a dose of 100 mg/kg dissolved in PBS. Mice were imaged before injection (T = 0) and 3, 6, 9, 12, 24, 48, and 72 h after injection.

Induction of CYP3A4-luc transgene with repeated dosing of three drugs. For repeated dosing experiments, mice in groups of three were injected i.p. with 100 μl of DMSO, or dexamethasone, rifampicin, or clotrimazole at 50 mg/kg. Mice were injected before injection (T = 0) and 3, 6, 9, 12, 24, 48, and 72 h after injection. Seven days after the first dosing, the mice were injected with the same compound at the same dose and were imaged again using the same time points.

In Vivo Imaging. In vivo bioluminescent imaging was performed as previously described (Contag et al., 1995). The substrate luciferin was injected into the intraperitoneal cavity at a dose of 150 mg/kg body weight (30 mg/ml luciferin) approximately 5 min before imaging. Mice were anesthetized with isoflurane/oxygen and placed on the imaging stage. Ventral and dorsal images were collected for 10 s to 1 min using the IVIS imaging system (Xenogen Corp., Alameda, CA). Photons emitted from the liver region were quantified using LivingImage software (Xenogen Corp.).
and brain were homogenized and sonicated using a tissue disrupter (Sonic Dismembrator 60; Fisher Scientific, Pittsburgh, PA) in 800 μl of PBS buffer. Luciferase activity was determined for 10-s integration using the Luciferase Assay System and a TD 20/20 Luminometer (Promega). Luciferase activities were normalized to relative light units per milligram of total protein in the homogenates. Protein content was measured using Bradford reagent (Sigma-Aldrich, St. Louis, MO).

Northern Analysis. Mouse liver, duodenum, kidney, spleen, lung, heart, and brain were excised and immediately frozen in liquid nitrogen. Total RNA from individual homogenates was extracted using RNeasy reagent (Ambion, Austin, TX). A single-strand antisense Cyp3a11 RNA probe was labeled using Strip-EZ RNA StripAble RNA Probe Synthesis and Removal Kit (Ambion). The RNA probe primarily detected the Cyp3a11 mRNA. However, since the RNA probe was synthesized from the entire Cyp3a11 coding region, it is possible that other Cyp3a isoforms could be detected with this probe. Three micrograms of total RNA from each homogenerate was loaded into each well. The blot was hybridized and detected following the instructions of the manufacturer. After detection, the blot was stained with 0.04% methylene blue in 0.5 M sodium acetate (pH 5.2) for 20 min and destained with 25% ethanol.

Western Analysis. Ten micrograms of protein from each tissue homogenerate were separated on a SDS-10% polyacrylamide gel and then transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA). Cyp3a protein level was detected using primary goat polyclonal antisera against rat CYP3A2 (BD Biosciences, Woburn, MA) and peroxidase-conjugated secondary antibody rabbit-anti-goat IgG (Sigma-Aldrich). This antibody is able to cross-react with human CYP3A4 and mouse Cyp3a proteins. Chemiluminescent reagents were purchased from Amersham Biosciences Inc.

Chemicals. DMSO, dexamethasone, and rifampicin, 5-pregnen-3β-ol-20-one, PCN, nifedipine, phenobarbital, and clotrimazole were purchased from Sigma-Aldrich.

Statistical Analysis. Data are presented in the text and figures as means ± standard error about the mean. Induction of the luciferase signal was analyzed by an analysis of variance with post hoc t tests to evaluate the difference between luciferase activity at the zero time point and each subsequent time point. To determine whether there was an overall difference in response to drug compared with either no injection or DMSO injection, a multivariate analysis of variance (MANOVA) model was used comparing the drug response for each drug with the response to vehicle. Male and female data were analyzed separately. Comparison of the first versus second response to either no injection, DMSO, dexamethasone, rifampicin, or clotrimazole injection was made using a MANOVA. Statistical tests were performed using the StatView statistical package (version 5.0.1; SAS Institute, Cary, NC).

Results

Founder Screening and Molecular Characterization. Transgenic mouse founders were identified by PCR detection of luciferase DNA in tail-tip DNA (Fig. 1B). Founder mice were backcrossed to FVB/N mice to generate progeny for screening as described under Materials and Methods. Nine founding lines were screened by in vivo imaging under baseline conditions and following induction by dexamethasone and rifampicin. The line with the highest baseline level of transgene expression in liver, and with the highest induction by dexamethasone and rifampicin, in both male and female mice was selected for full characterization as described in this report. Following standard genetic nomenclature rules, this strain has been named FVB/N-Tg(CYP3A4-luc)Xen. Genotyping by Southern hybridization of this founder was completed using luciferase cDNA as a probe. An expected 12-kb BamHI fragment containing 10 kb of the CYP3A4 promoter region and 2 kb of luciferase cDNA was observed in the heterozygous transgenic mouse genome (Fig. 1C).

Basal Expression of the CYP3A4-luc Reporter. To further characterize this transgenic line, we examined the basal expression pattern of the luciferase reporter by in vivo imaging of CYP3A4-luc mice. Six transgenic mice of each gender were injected with the substrate luciferin, and ventral and dorsal images were collected. In vivo imaging showed that male mice expressed the highest basal level of luciferase activity in the ventral upper abdomen and in the dorsal areas overlying the kidneys, whereas there was less significant luciferase activity in these regions in female mice (Fig. 2A). The signals quantified from the male ventral abdominal region were 16-fold higher than those from the female ventral abdomen, and male dorsal kidney region signals were 19-fold higher than the female dorsal signal (Fig. 2B).

To verify tissue distribution of luciferase activity, baseline luciferase activity in seven different organs, liver, duodenum, kidney, lung, spleen, heart, and brain, in both male and female mice at age of 6 to 8 weeks old (n = 3) was measured using a luminometer (Fig. 3A). In both genders, liver, duodenum, and kidney expressed the highest luciferase activity, whereas the other four tissues showed little luciferase activity. Male mice had higher luciferase activity than did female mice (101-, 59-, and 1.5-fold higher than female in liver, kidney, and duodenum, respectively). In male mice, liver and kidney...
had approximately equivalent basal luciferase activity, whereas duodenum luciferase activity was approximately 10% of that found in liver or kidney. In females, the duodenum had the highest luciferase activity, whereas the liver and kidney luciferase activity were 17% and 28% of the duodenum activity.

To compare the luciferase tissue distribution to the mouse Cyp3a mRNA and protein distribution, tissue samples were processed for Northern and Western analyses. A 1.5-kb Cyp3a11 antisense RNA probe was used to detect baseline Cyp3a mRNA. Liver and duodenum had the highest levels of Cyp3a mRNA, whereas expression in the other five tissues (including kidney) was not detectable. There were no significant differences in tissue mRNA levels between genders (Fig. 3B). When the same tissues were analyzed by anti-CYP3A2 antibodies, Cyp3a protein was found to be highest in liver and duodenum, and much lower in the other five tissues, in which signals were only detected with longer exposure (data not shown). There were no significant differences in tissue mRNA levels between genders (Fig. 3B). When the same tissues were analyzed by anti-CYP3A2 antibodies, Cyp3a protein was found to be highest in liver and duodenum, and much lower in the other five tissues, in which signals were only detected with longer exposure (data not shown). Apparently, Cyp3a protein in female liver was higher than that in male liver (Fig. 3C). In general, the levels of Cyp3a mRNA correlated with Cyp3a protein levels with no gender differences, even in livers.

In Vivo Drug Response of the Human CYP3A4 Promoter. We selected seven different CYP3A4 inducers including pregnenolone, phenobarbital, rifampicin, nifedipine, dexamethasone, PCN, and clotrimazole to study the effects of drugs on transcriptional regulation of the human CYP3A4 promoter. CYP3A4-luc mice, with males and females in separate groups, were challenged with different drugs at 50 mg/kg body weight except for phenobarbital at 100 mg/kg. DMSO was used as vehicle control, and a nontreated control group was also included. Mice were imaged from the ventral side at 0 (before injection) and 3, 6, 9, 12, 24, 48, and 72 h after a single administration of drug.

Injection of these drugs produced significant induction, to varying degrees, of the CYP3A4-luc transgene in vivo in both male (Fig. 4, A–C) and female (Fig. 5, A–C) mice. For all drugs, and in both genders, the peak luciferase induction occurred approximately 6 h after injection and returned to nearly baseline levels by 24 h after injection. Overall, there was greater induction by these drugs in male than in female mice.

For the male mice, relative to the DMSO response, all of the drugs tested in males except for pregnenolone produced a significant induction of luciferase signal (Fig. 4, A–C). The low inducers rifampicin and phenobarbital produced only a moderate luciferase induction at 6 h after injection and returned to nearly baseline levels by 24 h after injection. Overall, there was greater induction by these drugs in male than in female mice.

For all drugs tested in male CYP3A4-luc mice that induced the luciferase signal, the peak response occurred at 6 h after treatment (Fig. 4, B and C). For all drugs tested in male CYP3A4-luc mice that induced the luciferase signal, the peak response occurred at 6 h after treatment (Fig. 4, B and C).
In female mice, induction relative to DMSO was statistically significant for all of the drugs tested (Fig. 5). At 6 h after injection (Fig. 5, A and B), the low inducers, progesterone, phenobarbital, and rifampicin, induced luciferase 1.9- to 2.5-fold above the induction produced by DMSO. The highest inducer relative to DMSO was clomizamole, with a 14.9-fold induction at the 6-h time point. As with the male CYP3A4-luc mice, the response in the female mice generally peaked at 6 h after drug treatment (Fig. 5, B and C) and was not significantly different from DMSO control levels at 24 h after treatment.

In both genders, DMSO significantly induced the luciferase signal relative to baseline (3.4-fold for male; 3.2-fold for female; Figs. 4 and 5). In addition, in the nontreatment group the luciferase signal was significantly induced relative to baseline in the male mice (2.7-fold) but not in the female mice (1.3-fold). Since the drug vehicle (DMSO) and/or the anesthesia used during imaging produced a significant induction of the luciferase signal in the CYP3A4-luc mice, we concluded that the appropriate statistical analysis was to compare the drug effect with the DMSO group response.

**Induction of the Human CYP3A4-luc Reporter versus the Murine Cyp3a Genes.** Transgenic mice were treated with two prototypical CYP3A4 inducers, rifampicin and dexamethasone at 50 mg/kg, and the mice were imaged and sacrificed 6 h after drug administration. Liver tissues were processed for Northern analysis using a Cyp3a11 antisense RNA probe. Representative results are shown in Fig. 6. In male mice, rifampicin and dexamethasone induced both the CYP3A4-luc reporter and the endogenous Cyp3a genes in liver. Similarly in female mice, dexamethasone significantly induced both the transgene and the Cyp3a genes; however, the rifampicin treatment increased the luciferase reporter liver signal but had less effect on expression of the Cyp3a.

**In Vivo Response of the CYP3A4-luc Reporter to Repeated Drug Dosing.** We chose three compounds to assess whether the response to inducers of the CYP3A4-luc transgene changes with repeated dosing: rifampicin (low induction following a single dose), dexamethasone, and clomizamole (strong induction in the single-dose studies). Mice were first treated i.p. with these three compounds at 50 mg/kg body weight and imaged at various time points over 72 h. One week after the first dosing, the mice received the same drug at the same dose and were imaged. For controls, we used DMSO and a nontreated group. Generally, the induction of the CYP3A4-luc transgene after the second dosing had kinetic profiles similar to those of the first treatment in both genders (Fig. 7). At the peak time (T = 6 h after injection), the increase in luciferase signal in male mice relative to the zero time point was 2.4- and 3.5-fold for the first and second dosing, respectively, in the nontreated control groups, 3.2- and 4.3-fold in the DMSO control groups, 5.5- and 7-fold in the rifampicin-treated groups, 33- and 49.9-fold in the dexamethasone-treated groups, and 32-fold for both dosings in the clomizamole groups. In the females, there were 1.3- and 1.3-fold increases in the nontreated control group, 3- and 2.3-fold in the DMSO group, 11- and 8-fold in the rifampicin group, and 16-fold for both dosings in the dexamethasone group, and 22- and 15-fold in the clomizamole group. Comparing the response of the first week versus the second week to these three drugs in male mice by a multivariate analysis of variance, there was no difference in the first and second week response for either rifampicin or clomizamole. However, in the male CYP3A4-luc transgenic mouse, the induction by dexamethasone was significantly higher after the second dose compared with the first dose (MANOVA; p < 0.04). In female CYP3A4-luc mice, induction by dexamethasone and rifampicin was not different for the first and second week. However, in the female mice, the clomizamole response was significantly less in the second compared with the first week (MANOVA; p < 0.001).

**Discussion**

We examined the regulation of the human CYP3A4 promoter in a transgenic mouse model using a luciferase reporter. The human CYP3A4 promoter functioned in both female and male transgenic mice to regulate luciferase expression in response to various xenobiotics. This suggests that the mouse transcription factors can drive promoters for both the mouse Cyp3a and the human CYP3A4 gene. This is consistent with previous in vitro work demonstrating that the human CYP3A4 and rabbit CYP3A6 promoter activities were determined in part by the host cellular factors in primary cultures of adult rat and rabbit hepatocytes (Barwick et al., 1996). It is also in agreement with in vivo studies showing that the human CYP3A4 promoter was active in transiently transfected mouse livers (Schuett et al., 2002; Zhang et al., 2003).

The CYP3A4-luc transgenic animal model that we have developed allows us to follow induction of the transgene over time using the IVIS imaging system. Peak response was generally at 6 h after a single dose of drug treatment in both genders. One advantage of this model is that it is now possible to study the kinetics of CYP3A4 induction in vivo in real time. We found no comparable reports in the literature describing in detail the temporal response of the CYP3A4 gene to induction. It is difficult to compare the in vivo kinetics to the time course of induction in cell cultures because in the latter case, the inducer remains at a constant concentration in the culture medium, whereas in vivo, the drug must be transported to the tissues expressing CYP3A4, and concentration is time dependent.

The induction patterns of the CYP3A4-luc transgene in vivo by PCN, dexamethasone, rifampicin, and phenobarbital are similar to the reported activation profiles of mPXR (Barwick et al., 1996; Schuett et al., 2002; Zhang et al., 2003). However, responses to clomizamole and nifedipine are similar to hPXR activation patterns (Table 1; Quattrochoi and Guzelian, 2001). Strong induction of the human CYP3A4-luc reporter in vivo by the reportedly poor mPXR activator, clomizamole, suggests that other signaling pathways, such as constitutive androstane receptor (Wei et al., 2002), are involved. Pregnenolone has been previously described as a moderate activator for both human and murine PXR (Quattrochoi and Guzelian, 2001), but this drug had no effect on the transgene in males and negligible induction in females at the dose we used. It is possible that pregnenolone was metabolized.
rapidly in vivo and was not at a sufficient concentration to activate mPXR in mice.

In the line of CYP3A4-luc transgenic mouse described here, there were clear gender differences in both baseline luciferase signals and the level of induction, with basal signals and induction greater in male compared with female mice. We believe that this gender difference is not due to effects of the site of integration of the transgene. In three separate founding lines that were screened (data not shown), there was a consistent gender difference, with the male mice having higher basal and induced levels of the transgene. The gender differences in basal expression of the reporter are not in agreement with expression of the mouse Cyp3a genes (Figs. 3B and 6B), where the mRNA levels were comparable between the genders. Also, previous reports have shown that female mice express Cyp3a genes higher in liver and that the sexual dimorphism in hepatic Cyp3a expression in mice is regulated by the pattern of growth hormone regulation, ultimately activating the Janus kinase 2-signal transducer and activator of transcription 5 pathway (Udy et al., 1997; Sakuma et al., 2002). This could be a factor in our CYP3A4-luc transgenic model. However, the growth hormone regulation results in greater expression of Cyp3a in female mice, whereas in this transgenic model, the basal and induced level of the CYP3A4-luc transgene is less in females compared with males.

The literature is not consistent regarding gender differences in human CYP3A4 activity. Some studies have shown that CYP3A4 activity is higher in women than in men (Harris et al., 1995), but others described no major gender-specific differences in CYP3A activity (McCune et al., 2001; Meibohm et al., 2002). To explain the gender differences in the CYP3A4-luc transgene expression in transgenic mice, we speculate that androgen response element (ARE)-like sequences existing in the 5’ flanking region of CYP3A4 gene may be functionally activated by androgens in mice, but not in humans. Androgen receptor binds a wide range of imperfect palindrome sequences containing the core requirement of three of four guanines (underlined) within the ARE consensus sequence GGTACAnnnTGT-TCT (Nelson et al., 1999). Within the 13-kb upstream sequence of the CYP3A4 gene, no typical ARE elements were found. However, when compared with some known AREs from prostate-specific antigen and probasin promoters (Rennie et al., 1993; Huang et al., 1999), we found five potential AREs in the 5’ CYP3A4 sequence with 83% homology to other putative AREs found in other genes. The guanine residues in these DNA segments are conserved (Table 2). This speculation that the CYP3A4-luc transgene will respond to androgens was confirmed by implanting testosterone pellets subcutaneously into female CYP3A4-luc transgenic mice. Testosterone increased the CYP3A4-luc...
reporter activity by 9- and 28-fold in liver and kidney 9 days after implantation (data not shown).

The CYP3A4-luc transgene is expressed at the highest levels in liver and duodenum, which is in agreement with the endogenous mouse Cyp3a expression. However, the transgene expression in kidney was also relatively high in both males and females compared with the no detectable Cyp3a mRNA (Fig. 3, A and B) and faint Cyp3a protein (data not shown) in kidney. Previous reports showed that the CYP3A4 gene was polymorphically expressed in one of seven human kidneys (Schuetz et al., 1992), whereas the mouse Cyp3a11 and Cyp3a41 genes were expressed in mouse kidney at low levels (Sakuma et al., 2000). The unexpectedly high expression of the CYP3A4-luc transgene in mouse kidney could be due to the possibility that some DNA segments in the CYP3A4 promoter region mediate kidney-specific expression in mice. Although little is known about the molecular mechanisms of tissue-specific expression in kidney, a purine-rich GA element has been described which confers kidney-specific expression of rat chloride channel ClC-K1 gene. Purine-rich regions also have been found in the V2 vasopressin receptor and aquaporin-2 water channel promoters and are proposed to confer kidney-specific expression (Uchida et al., 1998). An analysis of the human CYP3A4 promoter revealed a purine-rich region, GGGGAGAGGAGAGTGGGGAGAAA (−9393 to −9417 nt) located at the distal 5′ region, whereas there was no such region found in the 11-kb mouse Cyp3a11 promoter. The kidney-specific activity mediated by this purine-rich region in the human CYP3A4 promoter appears to be stronger in mice than in humans.

The Cyp3a mRNA levels in seven different tissues correlated well with Cyp3a protein levels, with the highest mRNA content in liver, and a moderate mRNA quantity in duodenum. Despite discrepancies such as the relatively high level of expression in renal tissues, and gender differences in expression of the CYP3A4-luc transgene, some

![Fig. 7. Effects of repeated dosing on expression of the CYP3A4-luc transgene.](image)

A, time course of response in male mice following repeated drug injections. The second drug injection (indicated by the arrow) was given 7 days after the first injection at the same dose, 50 mg/kg body weight. Treatment groups are: nontreated control (NT), DMSO, rifampicin, dexamethasone, and clotrimazole. Data presented are for six mice per group and are depicted as group mean ± standard error. Photons were collected at time 0 and at 3, 6, 9, 12, and 24 h after the first drug injection. Mice also were imaged at 0 and at 3, 6, 9, 12, 24, 48, and 72 h after the second injection. Arrows indicate injections. B, the time course of response to repeated dosing in female mice depicted as in A.

### TABLE 1

Comparison of responses of CYP3A4-luc reporter in transgenic mice to activation of murine and human PXR in vitro

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<thead>
<tr>
<th>CYP3A4-luc transgenic mice</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
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<td>Rifampicin</td>
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<td>hPXR⁺</td>
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* Data summarized from a review by Quattrochi and Guzelian (2001).
inducers such as dexamethasone and rifampicin induced the reporter gene expression in a pattern very similar to the induction of the endogenous Cyp3a genes. This suggests that in mice there are similarities in the responses of the mouse promoters for the Cyp3a genes and the human CYP3A4 promoter to xenobiotics. This further confirms the previous result that the host cellular environment, along with the structure of the gene, dictates the pattern of CYP3A induction (Barwick et al., 1996).

The CYP3A4-luc transgene was induced by repeated imaging without injection of any drugs other than the luciferase substrate, luciferin, and isoflurane anesthesia. In preliminary studies, we have concluded that repeated isoflurane anesthesia and, perhaps, the injection of PBS (solvent for luciferin) are responsible for the slight induction of the endogenous mouse Cyp3a mRNA by DMSO. Other investigators have also reported that DMSO induces CYP3A4 in primary cultures of human hepatocytes in a concentration-dependent manner (LeCluyse et al., 2000). At a much lower dose (10%) of DMSO, others have reported no induction (Schuetz et al., 2000). The mechanism of induction of CYP3A4 by the high dose of DMSO is unclear. When using this in vivo system for evaluating agents that induce CYP3A4, it is important to always use concurrent vehicle and anesthesia controls.

The CYP3A4-luc transgenic model has both strengths and weaknesses when compared with in vitro primary human hepatocyte cultures, and hPXR-binding reporter assays, for screening CYP3A4 gene induction. If the purpose is to predict the response of the human CYP3A4 gene to a drug, then the results found in the CYP3A4-luc reporter mouse may not be entirely predictive since the induction pattern relies upon the mouse PXR and mouse transcription patterns. These differences in response may be due to differences in affinity for specific chemicals by human versus mouse PXR. Therefore, one may be able to detect induction of the luciferase reporter to agents that have low affinity for mouse PXR by testing higher doses. Furthermore, there are approaches for humanizing this model, including the replacement of the murine PXR with a human PXR (Xie et al., 2000), as well as humanizing other binding proteins and transcription factors. This would lead to a model more predictive of the human response.

Despite these limitations of the CYP3A4-luc model, there are also many advantages compared with studies using primary hepatocytes and with the PXR binding mediated in vitro models. Hepatocyte culture methods, and the PXR mediated in vitro models, provide no biodistribution information, and the kinetics of gene induction in vitro is not relevant to an in vivo environment. Our model is highly sensitive and allows one to follow the response of the same animals over time, providing a kinetic readout in vivo, although the kinetic profiles may vary significantly from those occurring in humans. For example, rifampicin activation of mPXR cannot be detected in reporter assays but is easily detectable in the CYP3A4-luc mouse. The readout by our in vivo model also provides information about bioavailability of the drug to the liver. The same animals potentially can be repeatedly dosed with various drugs, and the results of each administration can be monitored. Finally, probably the most significant advantage of this in vivo CYP3A4-luc model would be the ability to detect induction by metabolites that cannot be monitored in studies utilizing hepatoma cells and the CYP3A reporter assays. Therefore, the CYP3A4-luc model might be more efficient and effective for screening some aspects of CYP3A4 induction by drugs.

In summary, we describe here an in vivo transgenic mouse model, FVB/N-Tg(CYP3A4-luc)Xen, that allows us to examine the kinetics of transcriptional regulation of the CYP3A4 gene in vivo in real time. This model showed gender differences in basal and induction levels of the reporter. The response of this in vivo luciferase reporter system to drugs had similarities with the response of primary human hepatocytes to the same agents, but also showed some differences. This model provides an additional experimental approach for evaluating the transcriptional regulation of the CYP3A4 gene.

Acknowledgments. We thank Dr. Lidia Sambucetti for critical reading of the manuscript.

References

### Table 2

Possible AREs in the CYP3A4 gene

<table>
<thead>
<tr>
<th>Response Element Name (Location)</th>
<th>Putative CYP3A4 ARE</th>
<th>Reported ARE Elements in Other Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARE-1</td>
<td>GGAACAcTTGATT</td>
<td>GGAACAcTTGATT</td>
</tr>
<tr>
<td></td>
<td>~5042 To ~6956 nt</td>
<td>PSA Enhancer III</td>
</tr>
<tr>
<td>ARE-2</td>
<td>GGTCGAgcAGCCT</td>
<td>GGATCAGAgcAGTCT</td>
</tr>
<tr>
<td></td>
<td>~6667 To ~6681 nt</td>
<td>PSA ARE-2</td>
</tr>
<tr>
<td>ARE-3</td>
<td>AGAAAGGCGTCT</td>
<td>AGAAGAgcAGTCT</td>
</tr>
<tr>
<td></td>
<td>~1135 To ~1169 nt</td>
<td>PSA ARE-1</td>
</tr>
<tr>
<td>ARE-4</td>
<td>AGGTGAtaaAGCCTA</td>
<td>GGAACAtaaAGCCTA</td>
</tr>
<tr>
<td></td>
<td>~7777 To ~7791 nt</td>
<td>Probasin G-1</td>
</tr>
<tr>
<td>ARE-5</td>
<td>GGAACArTTGCAAC</td>
<td>GGAACArTTGCAAC</td>
</tr>
<tr>
<td></td>
<td>~9332 To ~9346 nt</td>
<td>PSA Enhancer V</td>
</tr>
</tbody>
</table>

nt, nucleotide.