DIFFERENTIAL REGULATION OF THE HUMAN CYP3A4 PROMOTER IN TRANSGENIC MICE AND RATS

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ABSTRACT:
Previously we described a transgenic mouse model [FVB/N-Tg(CYP3A4-luc)Xen] using a reporter construct consisting of 13 kilobases of the human CYP3A4 promoter driving the firefly luciferase gene in the inbred FVB/N mouse strain. Here we report regulation of the same CYP3A4-luc reporter gene in a transgenic outbred mouse strain (CD-1) and in a transgenic rat (Sprague-Dawley). Basal reporter expression and responses to several xenobiotics in the transgenic CD-1 mice [CD-1/Crl-Tg(CYP3A4-luc)Xen] were similar to those in the transgenic FVB/N mice. In both mouse backgrounds, the basal levels of the reporter were higher in male compared with female, and in the FVB/N strain there was greater induction for all drugs in male compared with female; however, in the CD-1 background this gender difference for induction was not obvious. In contrast with transgenic mice, transgenic rats [SD/Tac-Tg(CYP3A4-luc)Xen] expressed the luciferase reporter at higher basal levels in female compared with male rats. Responses to some compounds were much greater in rats than in mice, and the kinetics of induction was different with peak induction occurring later in the rat compared with the mouse. Our results suggest that the human CYP3A4 promoter is regulated differently in transgenic mice and rats in some aspects.

Modulation of cytochrome P450 (P4501) enzyme activities and induction of P450 gene expression in humans lead to changes in metabolism of therapeutic drugs, which can potentially result in a variety of adverse effects (Lin and Lu, 1998). Inhibition or activation of P450 enzymatic activity is usually a rapid process, whereas gene induction, which increases the total amount of enzyme and metabolic capacity, is relatively slower. Cytochrome P450 3A4 (CYP3A4) is one of the most important enzymes that metabolizes drugs. This gene is primarily expressed in liver and small intestine and is up-regulated by structurally diverse chemical compounds, thus increasing the capacity for metabolism of xenobiotics (Li et al., 1995). Increased expression of CYP3A4 is one factor that may produce adverse drug-drug interactions (Maurel, 1996). Current models for evaluating the regulation of P450 genes by xenobiotics include in vitro assays using primary hepatocytes, hepatoma cell lines, human pregnane X receptor (hPXR) activation of reporters, and in vivo animal models. An in vivo reporter assay in transiently transduced mouse liver for induction of CYP3A4-luc has also been reported (Schuetz et al., 2002; Zhang et al., 2003a). More recently, we described an in vivo transgenic CYP3A4-luc reporter in an inbred FVB/N mouse strain, allowing us to noninvasively monitor CYP3A4 induction kinetics (Zhang et al., 2003b).

The conventional rodent models used in toxicology and pharmacology are outbred rat strains that are genetically heterogeneous (Kacew, 2001). We have developed a CYP3A4-luc transgenic reporter in the Sprague-Dawley outbred rat strain. In addition, to compare the response of the CYP3A4-luc transgene in both an inbred and an outbred mouse strain, we have further created a CYP3A4-luc mouse in the outbred CD-1 mouse strain to complement the existing model in the FVB/N inbred strain that we have characterized (Zhang et al., 2003b). Having the same reporter in outbred mouse and outbred rat strains also allows us to compare species differences in the regulation of the human CYP3A4 promoter. Our results demonstrated that the human CYP3A4 promoter was regulated differently in mice and rats in basal and induced level of expression and kinetics of induction; only slight differences between outbred and inbred mouse strains were observed.

Materials and Methods
Generation of the CYP3A4-luc Transgenic Mouse and Rat Lines. The CYP3A4-luc reporter construct previously described by Zhang et al. (2003a) was used to make transgenic mice in outbred (CD-1) mice and Sprague-Dawley rats. This 15-kb DNA fragment containing a 13-kb CYP3A4 promoter, a chimeric intron, and the firefly luciferase cDNA was microinjected into single-cell stage Crl-CD-1 (ICR)BR (Charles River Laboratories, Inc., Wilmington, MA) mouse embryos and Tac:N(SD) Sprague-Dawley rat (Taconic Farms, Germantown, NY) embryos (Xenogen Biosciences, Cranbury, NJ). The injected embryos were implanted into pseudopregnant mice or rats. Transgenic founders and progeny were genotyped by PCR (Zhang et al., 2003b). Primary screening of transgenic lines was performed as previously described (Zhang et al., 2003b). Twelve independent CYP3A4-luc CD-1 mouse lines [CD-1/Crl-Tg(CYP3A4-luc)Xen] and 17 independent CYP3A4-luc SD rat lines [SD/Tac-Tg(CYP3A4-luc)Xen] were screened for their response to several known CYP3A4 inducers. One line from each species was selected for further char-
Fold induction of the CYP3A4-luc reporter in male (M) and female (F) transgenic mouse and rat

**TABLE 1**

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**Statistical Analysis.** Data are presented in the text and figures as means ± standard error about the mean. A multivariate linear equation was fit to the log(expression) data, where type of drug was entered as a dummy variable (DMSO always served as baseline). We used log(expression) because it provided parameters with a convenient interpretation, and the assumptions behind the statistical inference of the linear models used were not met using the raw expression data but were satisfied after the log-transformed data (e.g., symmetric residuals, constant variance). The exponentiated coefficients of these dummy variables in the linear models represented the relative expression of the drug versus DMSO. Standard errors were estimated using a one-term Taylor expansion. The models were used to compare the drug response for each drug with the response to vehicle, between species and genders. Female and male data were analyzed separately for CD-1 and FVB/N mouse strains. Female and male rat data were combined for analysis because there was no significant gender difference found between genders in the analysis.

**Results and Discussion**

**Xenobiotic Responses in CYP3A4-luc CD-1 Mice.** The basal expression levels of the CYP3A4-luc transgene and mouse CYP3A protein in seven different tissues in transgenic CD-1 mice (data not shown) were similar to those in FVB/N mice (for the FVB/N data, see Zhang et al., 2003b). In general, male CD-1 mice express higher basal luciferase activity than female in liver region (male, 29.0 vs. female, 1.27 photons/s x 107; p < 0.001). Pregnenolone showed little effect on reporter expression in either gender in either background strains, and, in general, both background strains and both genders responded most strongly to dexamethasone, PCN, and clotrimazole (Table 1). Statistical analysis showed that there was no significant difference in induction for each drug at the peak time between the CD-1 and FVB/N strains in both genders. Kinetics of induction in transgenic CD-1 mice and response to repeated dosing were also very similar to the transgenic FVB/N mice with peak at 6 h after treatment (data not shown). However, there was no significant gender difference in drug induction in transgenic CD-1 mice, whereas the FVB/N male mice responded more strongly to all drugs than female mice except for pregnenolone. The lack of gender difference in drug response in CD-1 mice could be strain-specific.

**Regulation of the CYP3A4-luc Reporter in Sprague-Dawley Transgenic Rats.** Female CYP3A4-luc transgenic rats had higher basal levels of reporter expression in liver than males (male, 6.91 ± 0.49; female, 33.1 ± 9.9; photons/s x 107; n = 3). This gender difference was characterized based upon appropriate anatomical distribution of expression and induction characteristics.

**Animal Studies and in Vivo Imaging.** Experiments were completed in CYP3A4-luc CD-1 mice and Sprague-Dawley rats at ages of 6 to 10 weeks old to assess induction of the CYP3A4-luc transgene by seven drugs (Zhang et al., 2003b). The seven drugs were dexamethasone, rifampicin, pregnenolone, 5-pregnen-3β-ol-20-one-16α-carbonitrile (PCN), nifedipine, phenobarbital, and clotrimazole (Sigma-Aldrich, St. Louis, MO). Dimethyl sulfoxide (DMSO) was used as the vehicle. Nontreated groups were also included. Doses of the drug versus DMSO. Standard errors were estimated using a one-term Taylor expansion. The models were used to compare the drug response for each drug with the response to vehicle, between species and genders. Female and male data were analyzed separately for CD-1 and FVB/N mouse strains. Female and male rat data were combined for analysis because there was no significant gender difference found between genders in the analysis.
The difference is opposite to that observed in the mouse lines (Fig. 1A). Distribution of the transgene and the endogenous rat CYP3A proteins in liver, duodenum, kidney, lung, spleen, heart, and brain in both male and female rats at the ages of 6 to 8 weeks were analyzed by luminometer and by Western blot (Fig. 1B). Liver, kidney, and duodenum had high luciferase levels, whereas the other four tissues had very low levels. Luciferase activity in the female liver was approximately 3.9-fold higher than that in the male liver, consistent with the gender difference observed by in vivo imaging. The kidney signals were comparable in both genders, although the duodenum signal was higher in male than in female rats. Using a polyclonal antiserum against the rat CYP3A2 isoform, we detected CYP3A signals in liver, duodenum, kidney, and spleen in both genders by Western blotting, and the signals in the liver were significantly higher than in other tissues in both genders. The signal in male liver was slightly higher than in female liver, consistent with a previous study that used the same antibody (Johnson et al., 2000). However, the strong signal observed in female liver does not agree with the fact that the CYP3A2 is male-specific in adult rats (Cooper et al., 1993). Since this CYP3A2 polyclonal antibody has not been rigorously tested for cross-reactivity with other rat CYP3A forms, it is clear that this antibody is not measuring specifically the CYP3A2 levels. Therefore, the Western results can only refer to general CYP3A protein levels.

The higher basal expression level of the luciferase reporter in female liver compared with male liver in CYP3A4-luc transgenic rats was not consistent with the gender difference in the CYP3A protein levels detected by Western blotting. This higher luciferase expression in female rats (3.9-fold higher than in males) likely mirrored in part the female-dominant CYP3A9 that is expressed 28 times higher in female than in male (Anakk et al., 2003). The opposite gender differences for the reporter in rat versus mice are likely due to species differences in the transcriptional regulation of the reporter and not due to insertional effects. This conclusion is supported by the observations made in screening multiple founder lines (minimum of three) for both the CD-1 and FVB/N mouse background strains. In the majority of mouse founder CD-1 and FVB/N strains that were screened, the basal levels of transgene expression were higher in male compared with female mice. However, when multiple SD rat transgenic lines were screened, the female rats consistently had higher expression levels of the transgene compared with the male rats. Higher expression of the CYP3A4-luc reporter in female rats could be due to the fact that there are three putative estrogen-response elements located in the proximal promoter region of the human CYP3A4 gene which could be active in the rat but not the mouse (Hashimoto et al., 1993). The androgen-response-like elements that are also found in the human CYP3A4 promoter may not be functional in rats (Zhang et al., 2003b).

Injection of seven different drugs into the CYP3A4-luc rats produced marked induction of the transgene to various degrees in vivo in...
both genders with three animals tested for each gender (Fig. 2). Female CYP3A4-luc rats tended to be more responsive to these compounds than were male rats, which was opposite to the gender differences found in the mouse. However, statistical analysis showed no significant gender difference with relatively small residual variance (three animals per gender). Therefore, we combined data from three females and three males per group for statistical analysis. The peak luciferase induction occurred approximately 12 to 30 h after injection (Fig. 2B), which is significantly delayed compared with either mouse strain carrying the CYP3A4-luc reporter where the peak response occurred at 6 h (Zhang et al., 2003b). The signals remained high for a longer period of time in the rat compared with the mouse, especially for dexamethasone and PCN (Fig. 2B). This longer induction kinetics compared with mouse could be related to differences in absorption, excretion, and metabolism of the drugs, or it could be attributed to different kinetics of gene induction. As in mice, dexamethasone, clotrimazole, and PCN produced the greatest transgene induction; phenobarbital and nifedipine were moderate inducers, whereas rifampicin and pregnenolone produced minimal induction.

These in vivo induction response data in the CYP3A4-luc transgenic rats are consistent with a report using rat primary hepatocyte cultures (Kocarek et al., 1995). Surprisingly, we observed that the levels of induction by dexamethasone, clotrimazole, PCN, phenobarbital, and nifedipine were much higher in rats than in mice (Table 1) and higher than observed in rat hepatocytes (Kocarek et al., 1995). This comparison of fold induction between species may not be accurate because we used the same doses in both rats and mice, and the plasma profiles of each compound in either species were not measured. Therefore, species difference in fold induction could be highly dependent on plasma levels of these compounds. However, the data estimated relative effects of these drugs on the CYP3A4-luc reporter in different species. Greater induction in rats suggests that rat receptors may have higher binding affinity to the CYP3A4 promoter. It has been reported that clotrimazole and phenobarbital produced very little induction of a reporter construct using a 1-kb CYP3A4 promoter in rat hepatocytes (Xie et al., 2000), and that these two compounds activated the rat or mouse PXR poorly in CV-1 cells (Jones et al., 2000). Strong induction by clotrimazole and moderate induction by phenobarbital in CYP3A4-luc rats (Table 1) suggest that other signaling pathways such as constitutive androstane receptor-mediated induction may be involved for these two drugs (Wei et al., 2002). It is also possible that additional response elements may exist in the −1.1- to −13-kb region of the CYP3A4 promoter we used in this transgenic model or may reflect real differences between in vivo and in vitro measurements. The patterns of relative effects of these seven compounds in transgenic rats exhibited the same profiles as in the two transgenic mouse strains (Table 2). This suggests that rats and mice may share similar xenobiotic signaling pathways including pregnane X receptor and constitutive androstane receptors (Jones et al., 2000; Wei et al., 2002). When we compared relative effects of these compounds with in vitro activation of human, rat, and mouse PXRs (Table 2), our data indicate that this rat model mimics the rat PXR response rather than the human PXR except for clotrimazole and phenobarbital. These data also further confirmed previous studies that the host cellular factors in different species dictate the regulation of the CYP3A genes (Barwick et al., 1996). Therefore, the CYP3A4-luc reporter rats as well as the transgenic mice may not be entirely predictive for human CYP3A4 regulation since the induction pattern relies upon the rat PXR and rat activation patterns. One may be able to detect induction of the luciferase reporter to agents that have low affinity for rat PXR by...
testing higher doses. Further humanizing this model by the replacement of the rat PXR with a human PXR (Xie et al., 2000), as well as humanizing other binding proteins and transcription factors, may make the model more predictive of the human response.

In summary, we have shown that the CYP3A4-luc transgene regulation is different in rats versus mice, although the effect of background strain on transgene regulation in mice is minimal. In rats the induction of the reporter is greater than in mice, and the induction kinetics are quite different between species. However, in general the responsiveness to different chemical agents is similar in the two species. Although the response of the transgene is significantly affected by the presence of the rodent binding proteins, the potential exists for further humanizing this model system to better predict human response to xenobiotics. The advantages of using bioluminescence to track and monitor the drug response include the ability to follow the response of the same animal over time noninvasively and in vivo, while animal systems have significant advantages over in vitro approaches for studying induction.

**References**


